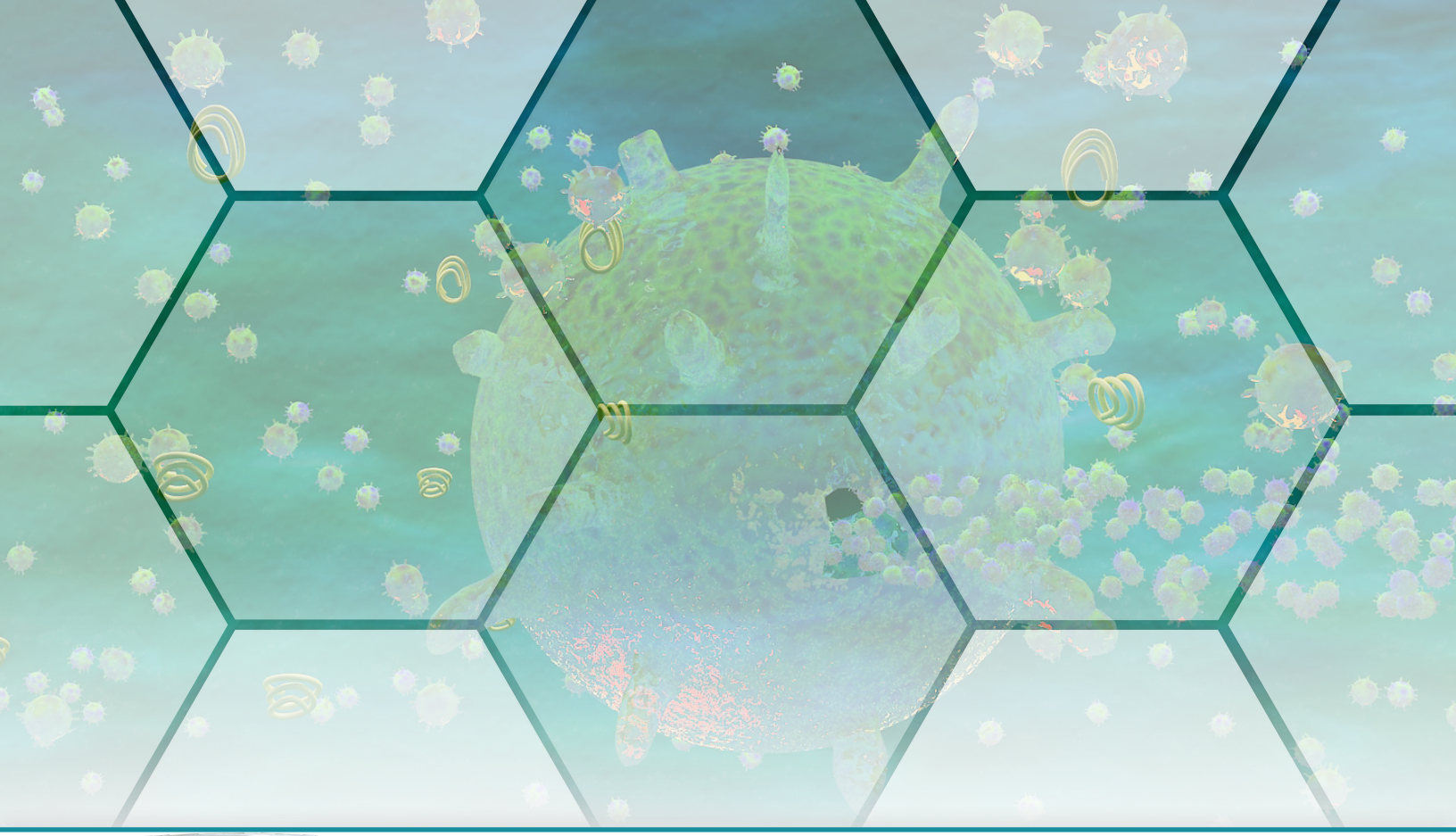


EXOSOMES

Exosome Purification, Fractionation of Exosomal Free & Circulating RNA from Bodily Fluids

NORGEN BIOTEK CORP.

CERTIFIED QUALITY ISO 9001:2015 CERTIFIED QUALITY

CERTIFIED QUALITY ISO 13485:2016 CERTIFIED QUALITY

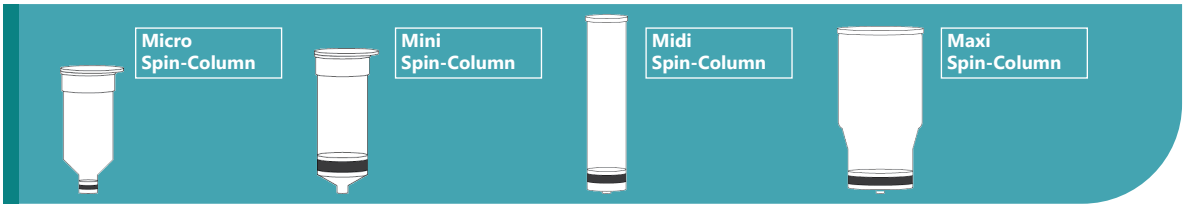
About Norgen

Our capabilities will meet every step of your workflow.

Norgen Biotek is dedicated to providing our customers with first class sample preparation kits for RNA, microRNA, DNA, and protein purification, clean-up and concentration for research and diagnostic applications; and to provide dedicated and expert support services to our customers and commercial partners worldwide. **Our products and services span the complete workflow from sample collection and preservation to purification and analysis.**

Norgen is an **ISO 9001: 2015** and **ISO 13485: 2016** registered company, indicating our commitment to quality.

Our column-based kits offer a versatile sample input range



Product pages indicate available sizes

Ordering Information

To Order by Phone:
Tel: (905) 227-8848
Toll Free in North America: 1-866-667-4362

To Order by Fax:
(905) 227-1061

To Order by Email:
orders@norgenbiotek.com

To Order by Mail:
Norgen Biotek Corp.
3430 Schmon Parkway
Thorold, Ontario
L2V 4Y6
CANADA

v3.0

Background What is an Exosome?

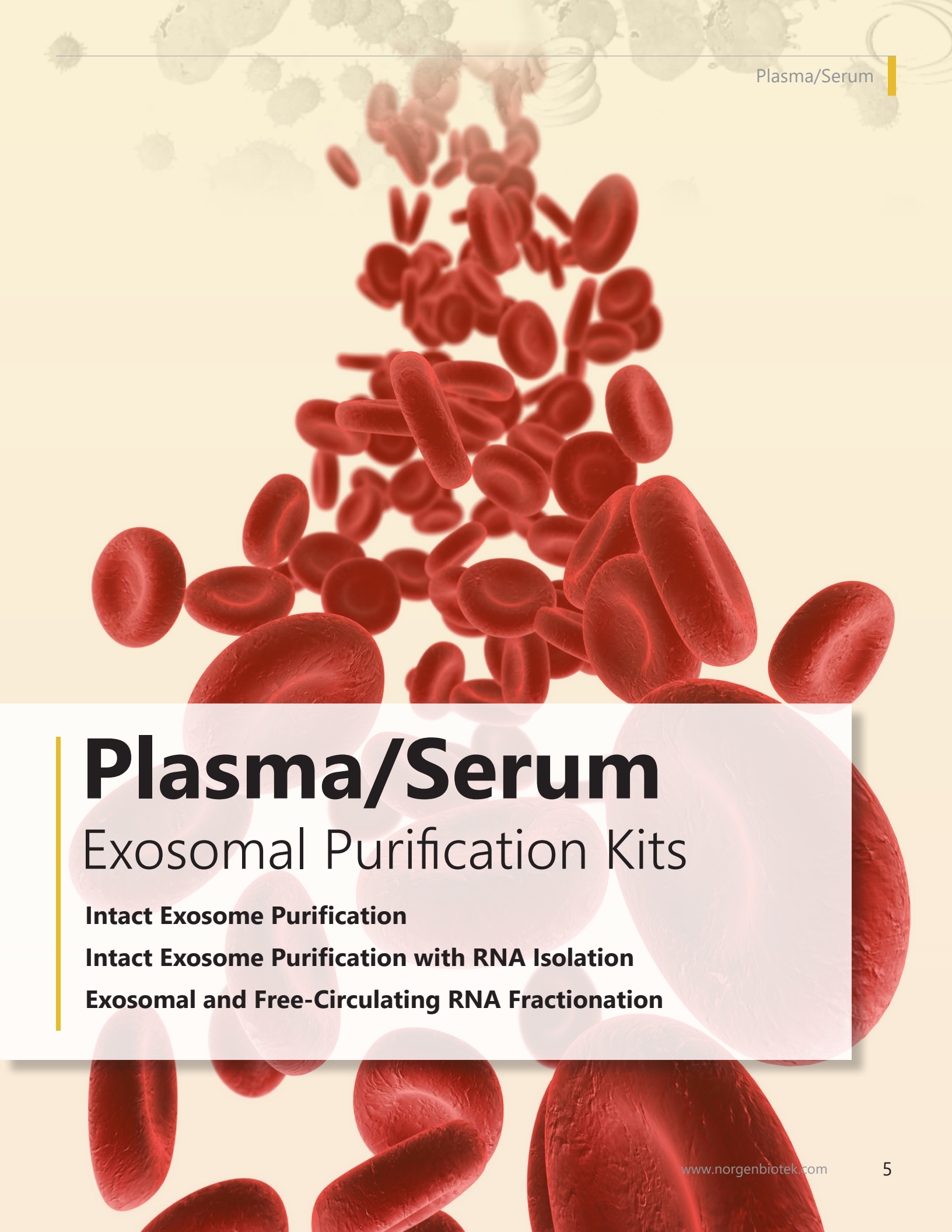
Exosomes are 40 - 150 nm membrane vesicles which are secreted by most cell types. Exosomes can be found in cell culture media, plasma, serum, saliva, urine, amniotic fluid, and malignant ascite fluids, among other biological fluids.

Recent evidence has shown that these vesicles act as cellular messengers, conveying information to distant cells and tissues within the body. The exosomes contain cell-specific proteins, lipids and RNAs, which are transported to other cells, where they can alter function and/or physiology. These exosomes may play a functional role in mediating adaptive immune responses to infectious agents and tumours, tissue repair, neural communication, and transfer of pathogenic proteins.

Recent work has demonstrated the presence of distinct subsets of microRNAs within exosomes and other extracellular vesicles (EVs) which depend upon the tumour cell type from which they are secreted. For this reason exosomal RNA may serve as biomarkers for various diseases including cancer.

Another subset of RNA found in bodily fluids and cell culture media is the free-circulating RNA (fc-RNA). These fc-RNA are usually protein-bound RNA that are leaked from cells either during apoptosis or necrosis. As the RNA molecules encapsulated within exosomes or bound to proteins (fc-RNA) are protected from degradation by RNAses, they can be efficiently recovered from bodily fluids and cell culture media. In general, these two RNA groups contain valuable information for the discovery of biomarkers that can help with early detection of certain cancer types and for monitoring the disease status.

Norgen has developed a comprehensive basket of kits that constitute an all-in-one system for the purification, isolation, and fractionation of exosomal RNA and free-circulation RNA from plasma, serum, ascitic fluid, urine, saliva, and cell culture media. As the RNA molecules encapsulated within exosomes are protected from degradation by RNAses they can be efficiently recovered from biological fluids. Norgen's kits, therefore, make exosomal RNA discovery simple, rapid, and reliable. Users can simultaneously concentrate and isolate high quality exosomal RNA, including microRNA, for use in sensitive downstream assays.



Plasma/Serum

Exosomal Purification Kits

Intact Exosome Purification

Intact Exosome Purification with RNA Isolation

Exosomal and Free-Circulating RNA Fractionation

Plasma/Serum Exosome Purification Kits

Cat No. 57400, 57500, 57600

- Purification and enrichment of intact plasma/serum exosomes for functional studies
- Versatile Sample input range
- No time-consuming ultracentrifugation, filtration nor special syringes required
- No precipitation reagents nor overnight incubation required
- Compatible with plasma/serum from any species
- Pure exosomes are purified and are free-from any other RNA-binding proteins

Rapid and simple purification of **intact exosomes** from plasma/serum samples

Norgen's Plasma/Serum Exosome Purification Kits constitute an all-in-one system for the purification of exosomes from different plasma/serum sample volumes ranging from 50 µL up to 10 mL. These kits also allow for the purification of intact extracellular vesicles (EVs) from different plasma/serum sample volumes, and these EVs are ready for any downstream application. The purification is based on Norgen's proprietary resin. These kits provide a clear advantage over other available kits in that they do not require any special instrumentation, precipitation reagents or any protease treatments. More importantly, the purified exosomes will not be contaminated with any other RNA-bound proteins that may contaminate your exosomal RNA, which is essential if studying Exosomal RNA gene expression.

Versatile sample input ranging from 50 µL - 10 mL

Feature	Specification
Mini Kit Input Range	50 µL - 1 mL
Midi Kit Input Range	1 mL - 4 mL
Maxi Kit Input Range	4 mL - 10 mL
Size of Exosomes Purified	40 nm - 150 nm
Time to Complete 10 Purifications	15 - 30 minutes

Ordering Information

Description	Size	Cat. Number
Plasma/Serum Exosome Purification Mini Kit	50 preps	57400
Plasma/Serum Exosome Purification Midi Kit	25 preps	57500
Plasma/Serum Exosome Purification Maxi Kit	15 preps	57600

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
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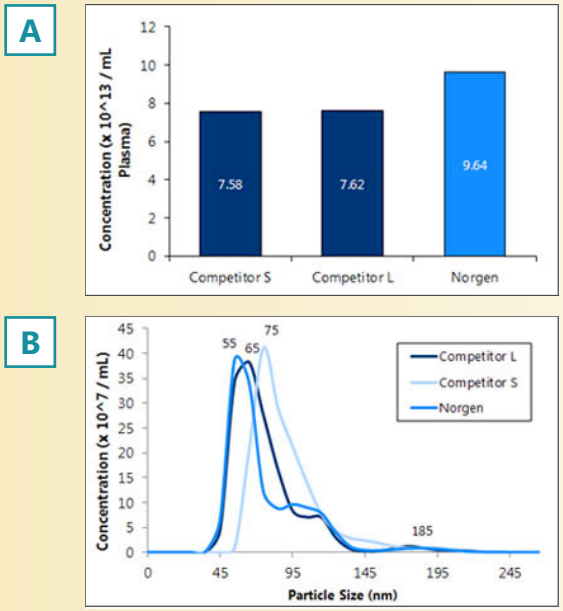

Figure 1. Intact exosomes were purified from 1 mL plasma using different purification methods. Intact exosomes were purified from 1 mL plasma using Norgen's Plasma/Serum Exosome Purification Mini Kit (Cat# 57400), Competitor S's kit and Competitor L's kit (from plasma). Exosomes purified using Norgen's kit were resuspended in 200 µL of Norgen's ExoR buffer, diluted 1:1,000 and visualized on the NanoSight LM10 instrument. The analysis shows that Norgen's kit isolated 55 nm exosomes with a recovery of 9.64 x 1013 particles/mL plasma. No impurities were found to be contaminating the exosomes purified using Norgen's Plasma/Serum Exosome Purification Mini Kit. Additionally, exosomes with a broader size range covering from 50 nm - 150 nm were purified from 1 mL plasma with a higher concentration compared to the other two methods.

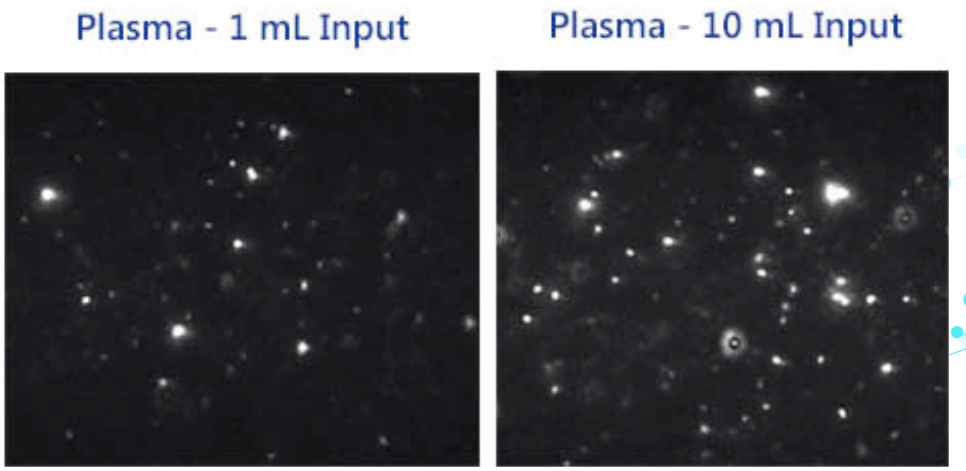

Figure 2. Intact exosomes purified from 1 mL and 10 mL plasma. Intact exosomes were purified from 1 mL plasma using Norgen's Plasma/Serum Exosome Purification Mini Kit (Cat # 57400) and from 10 mL plasma using Norgen's Plasma/Serum Exosome Purification Maxi Kit (Cat # 57600). Exosomes purified using Norgen's Mini kit were resuspended in 200 µL of Norgen's ExoR buffer whereas exosomes purified using Norgen's Maxi kit were resuspended in 600 µL Norgen's ExoR buffer, diluted 1:1,000 and visualized on the NanoSight LM10 instrument. The analysis shows that the purification of exosomes is linear as 4.04 x 1010 particles/mL was recovered from 1 mL plasma whereas 2.95 x 1011 particles/mL was recovered from 10 mL plasma.

Plasma/Serum Exosome Purification and RNA Isolation Kits

Cat No. 58300, 58500, 58600

- Purification and enrichment of intact plasma/serum exosomes for functional studies
- Bind and elute all RNA irrespective of size or GC content, without bias
- Isolate all sizes of exosomal and extracellular vesicle RNA, including microRNA
- Versatile plasma/serum input volume (50 µL - 10 mL)
- No phenol extractions, Proteinase K treatment, nor carrier RNA required
- No time-consuming ultracentrifugation, filtration nor special syringes required
- No precipitation reagents, nor overnight incubation required

Rapid and simple purification of **intact exosomes and RNA isolation** from plasma/serum samples

Norgen's Plasma/Serum Exosome Purification and RNA Isolation Kits constitute an all-in-one system for the purification of exosomes and the subsequent isolation of exosomal RNA from different plasma/serum sample volumes ranging from 50 µL to 10 mL. The purification is based on spin-column chromatography that employs Norgen's proprietary resin. The kit is designed to isolate all sizes of extracellular vesicle RNA, including microRNA. The kit provides a clear advantage over other available kits in that they do not require any special instrumentation, protein precipitation reagents, extension tubes, phenol/chloroform or protease treatments. Moreover, the kit allows the user to elute into a flexible elution volume ranging from 50 µL to 100 µL. The purified RNA is free from any protein-bound circulating RNA and is of the highest integrity. The purified RNA can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Versatile sample input ranging from 50 µL - 10 mL

Feature	Specification
Mini Kit Input Range	50 µL - 1 mL
Midi Kit Input Range	1 mL - 4 mL
Maxi Kit Input Range	4 mL - 10 mL
Size of Exosomes Purified	40 nm - 150 nm
Time to Complete 10 Purifications	15 - 40 minutes

Ordering Information

Description	Size	Cat. Number
Plasma/Serum Exosome Purification and RNA Isolation Mini Kit	50 preps	58300
Plasma/Serum Exosome Purification and RNA Isolation Midi Kit	25 preps	58500
Plasma/Serum Exosome Purification and RNA Isolation Maxi Kit	15 preps	58600

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

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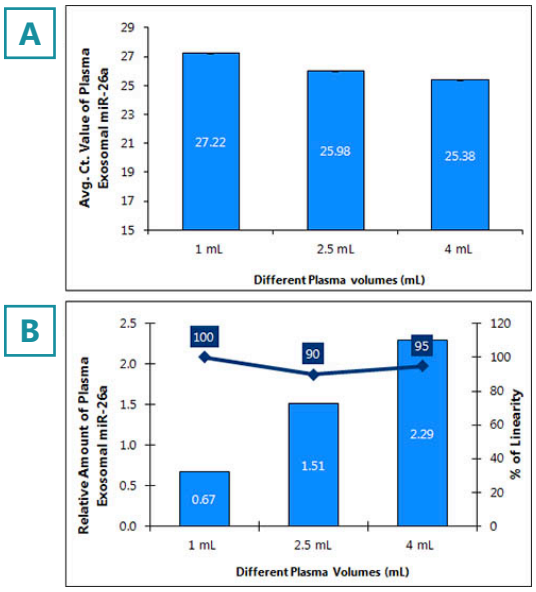

Figure 1. Isolation of RNA from exosomes purified from different plasma volumes. Norgen's Plasma/Serum Exosome Purification and RNA Isolation Midi Kit (Cat# 58500) was used to isolate RNA from exosomes purified from different plasma volumes using the same kit. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated plasma exosomal miR-26a. (A) The plasma exosomal miR-26a is linearly decreasing with increasing sample input volume. B) The relative amount of the plasma exosomal miR-26a shows excellent linearity with a percentage of recovery of more than 90%.

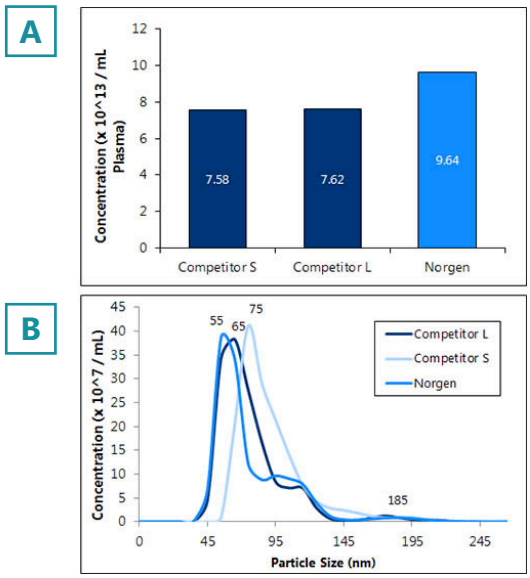

Figure 2. Intact exosomes were purified from 1 mL plasma using different purification methods. Intact exosomes were purified from 1 mL plasma using Norgen's Plasma/Serum Exosome Purification and RNA Isolation Mini Kit (Cat# 58300), Competitor S's kit and Competitor L's kit (from plasma). Exosomes purified using Norgen's kit were resuspended in 200 µL of Norgen's ExoR buffer, diluted 1:1,000 and visualized on the NanoSight LM10 instrument. The analysis shows that Norgen's kit isolated 55 nm exosomes with a recovery of 9.64 x 10^13 particles/mL plasma. No impurities were found to be contaminating the exosomes purified using Norgen's Plasma/Serum Exosome Purification and RNA Isolation Mini Kit. Additionally, exosomes with a broader size range covering from 50 nm - 150 nm was purified from 1 mL plasma with a higher concentration compared to the other two methods.

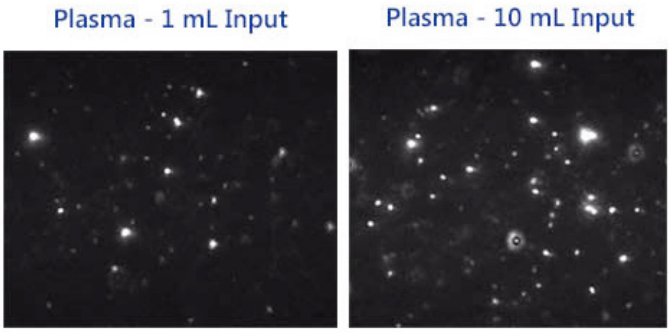

Figure 3. Intact exosomes purified from 1 mL and 10 mL plasma. Intact exosomes were purified from 1 mL plasma using Norgen's Plasma/Serum Exosome Purification and RNA Isolation Mini Kit (Cat# 58300) and from 10 mL plasma using Norgen's Plasma/Serum Exosome Purification and RNA Isolation Maxi Kit (Cat# 58600). Exosomes purified using Norgen's mini kit were resuspended in 200 µL of Norgen's ExoR buffer, whereas exosomes purified using Norgen's Maxi kit were resuspended in 600 µL Norgen's ExoR buffer, diluted 1:1,000 and visualized on the NanoSight LM10 instrument. The analysis shows that the purification of exosomes is linear as 4.04 x 10^10 particles/mL were recovered from 1 mL plasma whereas 2.95 x 10^11 particles/mL were recovered from 10 mL plasma.

Plasma/Serum Exosome and Free-Circulating RNA Fractionation Kits

Cat No. 59500, 59600, 59700

- Isolate all sizes of exosomal and extracellular vesicle RNA, including microRNA as well as free-circulating RNA, including microRNA
- Bind and elute all RNA irrespective of size or GC content, without bias
- The purified exosomal RNA is free from any circulating RNA-binding proteins
- No phenol extractions, Proteinase K treatment, or carrier RNA
- No time-consuming ultracentrifugation, filtration nor special syringes are required
- No precipitation reagents nor overnight incubation required
- Concentrate isolated exosomal RNA and free-circulating RNA into a flexible elution volume ranging from 50 µL to 100 µL

Rapid and simple sequential isolation of **exosomal** and **free-circulating RNA** from different plasma/serum sample volumes

Norgen's Plasma/Serum Exosome and Free-Circulating RNA Isolation Kits constitute an all-in-one system for the purification of exosomes and the sequential isolation of exosomal RNA and free-circulating RNA from different plasma/serum sample volumes ranging from 1 mL to 4 mL. The purification is based on spin-column chromatography that employs Norgen's proprietary resin. The kit is designed to isolate all sizes of extracellular vesicle RNA, including microRNA. The kit provides a clear advantage over other available kits in that they do not require any special instrumentation, protein precipitation reagents, extension tubes, phenol/chloroform or protease treatments. Moreover, the kit allows the user to elute into a flexible elution volume ranging from 50 µL to 100 µL. The RNA isolated from the purified exosomes is free from any proteinbound circulating RNA and is of the highest integrity. Moreover, the free-circulating, protein-bound, RNA is free from any exosomal RNA. The purified RNA can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Versatile sample input ranging from 50 µL - 10 mL

Feature	Specification
Mini Kit Input Range	50 µL - 1 mL
Midi Kit Input Range	1 mL - 4 mL
Maxi Kit Input Range	4 mL - 10 mL
Size of Exosomes Purified	40 nm - 150 nm
Time to Complete 10 Purifications	15 - 40 minutes

Ordering Information

Description	Size	Cat. Number
Plasma/Serum Exosome and Free-Circulating RNA Isolation Mini Kit	50 preps	59500
Plasma/Serum Exosome and Free-Circulating RNA Isolation Midi Kit	25 preps	59600
Plasma/Serum Exosome and Free-Circulating RNA Isolation Maxi Kit	15 preps	59700

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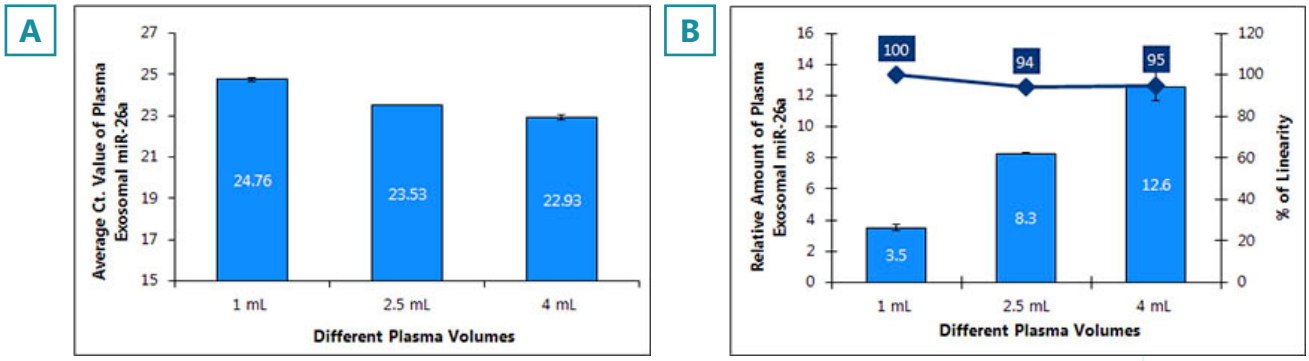

Figure 1. Isolation of RNA from exosomes purified from different plasma volumes. Norgen's Plasma/Serum Exosome and Free-Circulating RNA Isolation Midi Kit (Cat# 59600) was used to isolate Exosomal RNA from different plasma volumes ranging from 1 mL and up to 4 mL. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated plasma exosomal miR-26a. (A) The plasma exosomal miR-26a is linearly decreasing with increasing the sample input volume. B) The relative amount of the plasma exosomal miR-26a shows excellent linearity with a percentage of recovery of more than 90%.

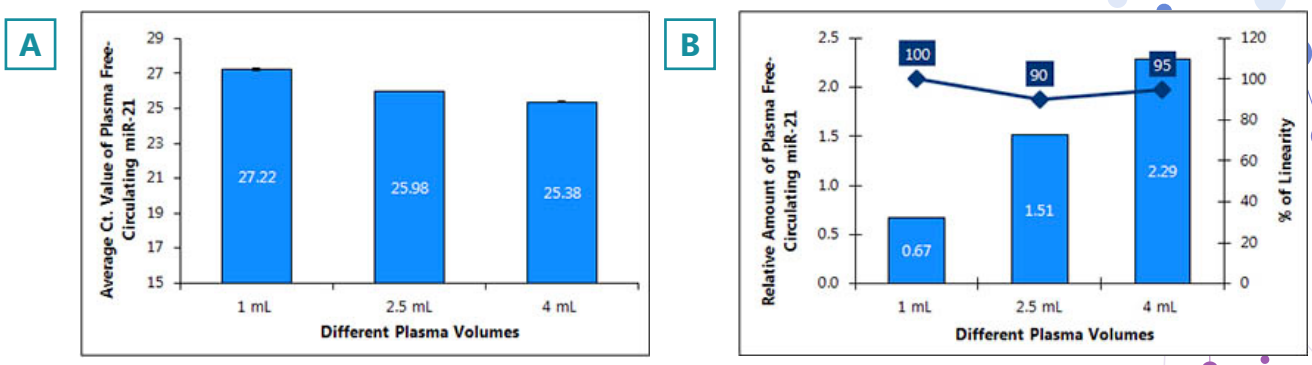

Figure 2. Isolation of Free-Circulating RNA after the isolation of Exosomal RNA from different plasma volumes. Norgen's Plasma/Serum Exosome and Free-Circulating RNA Isolation Midi Kit (Cat# 59600) was used to isolate Free-Circulating RNA after the isolation of Exosomal RNA from different plasma volumes ranging from 1 mL and up to 4 mL. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated plasma free-circulating miR-21. (A) The free-circulating miR-21 is linearly decreasing with increasing the sample input volume. B) The relative amount of the free-circulating miR-21 shows excellent linearity with a percentage of recovery of more than 90%.

Select Publication

Using Norgen's Plasma/Serum Exosome Purification and RNA Isolation Kits

RNA-Sequencing Analysis of Human Plasma-Derived Extracellular Vesicles as Potential Circulating Biomarkers in Chronic Obstructive Pulmonary Disease

I. Sundar[1], D. Li[2], I. Rahman[3];

[1]Dept of Environmental Medicine, University of Rochester, Rochester, NY, United States,
[2]Clinical and Translational Research and Public Health Sciences, University of Rochester, Rochester, NY, United States,
[3]Univ of Rochester, Rochester, NY, United States.

B61. COPD: BASIC DISCOVERY. May 1, 2019, A3787-A3787

Rationale: Extracellular vesicles (EVs) play a vital role in normal lung physiology to maintain homeostasis in the airways via intercellular communication. Exosomes (nano-sized vesicles: 50-150 nm in diameter) are characterized by their endosomal origin, released by different cell types such as epithelial, fibroblasts, endothelial, tumor cells, stem cells including immune inflammatory cells. In this study, we characterized the small-RNAs from plasma-derived exosomes samples of non-smokers, smokers and patients with COPD as EV biomarkers. Methods: Human plasma-derived exosomes from non-smokers, smokers and patients with COPD were isolated using Exosome purification and RNA isolation kit (Norgen Biotek, ON, Canada). Further characterization of EVs based on their size was performed using nanoparticle tracking analysis (NTA) or tunable resistive pulse sensing by Nanosight or qNano, transmission electron microscopy (TEM), and EV surface markers by immunoblot analysis. RNA isolated from EVs were used for small RNA library preparation and RNA sequencing (RNA-seq). DESeq2 method was used for differential expression analysis of RNA-seq data to identify significantly increased or decreased miRNAs. Gene enrichment analysis was conducted using Funrich and Ingenuity pathway analysis (IPA). Results: We found that plasma-derived EVs from non-smokers, smokers and patients with COPD vary in their size, concentration, distribution and phenotypic characteristics as confirmed by NTA, TEM, and EV surface markers. RNA-seq analysis identified most abundant small RNAs, such as miRNAs, tRNAs, piRNAs and circular RNAs which have diverse functions. We further focused on characterizing miRNAs as biomarkers of disease. DESeq2 analysis revealed differentially expressed miRNA targets in non-smokers versus smokers versus COPD (increased: miR-22-3p, miR-99a-5p, miR-151a-5p, miR-320b) and (decreased: miR-335-5p, miR887-5p, miR320d, miR937-3p and miR-628-3p). Gene enrichment analysis of miRNA targets revealed top biological processes (cell-cell communication, signal transduction and regulation of nucleic acid metabolism) and top pathways (Glypican pathway, Integrin family and Beta 1 integrin cell surface interaction, and TRAIL signaling) associated with smokers and patients with COPD compared to non-smokers. We are currently validating selected miRNA targets in EVs isolated from human bronchial epithelial cells treated with cigarette smoke. Conclusion: We identified selected miRNAs in systemic exosomes with their target biological pathways, which are distinct in COPD with and without smokers. Identifications of miRNAs has a great translational potential for the development of novel miRNA signatures from EVs as biomarkers that may be used in the diagnosis, prognosis and therapeutics of patients

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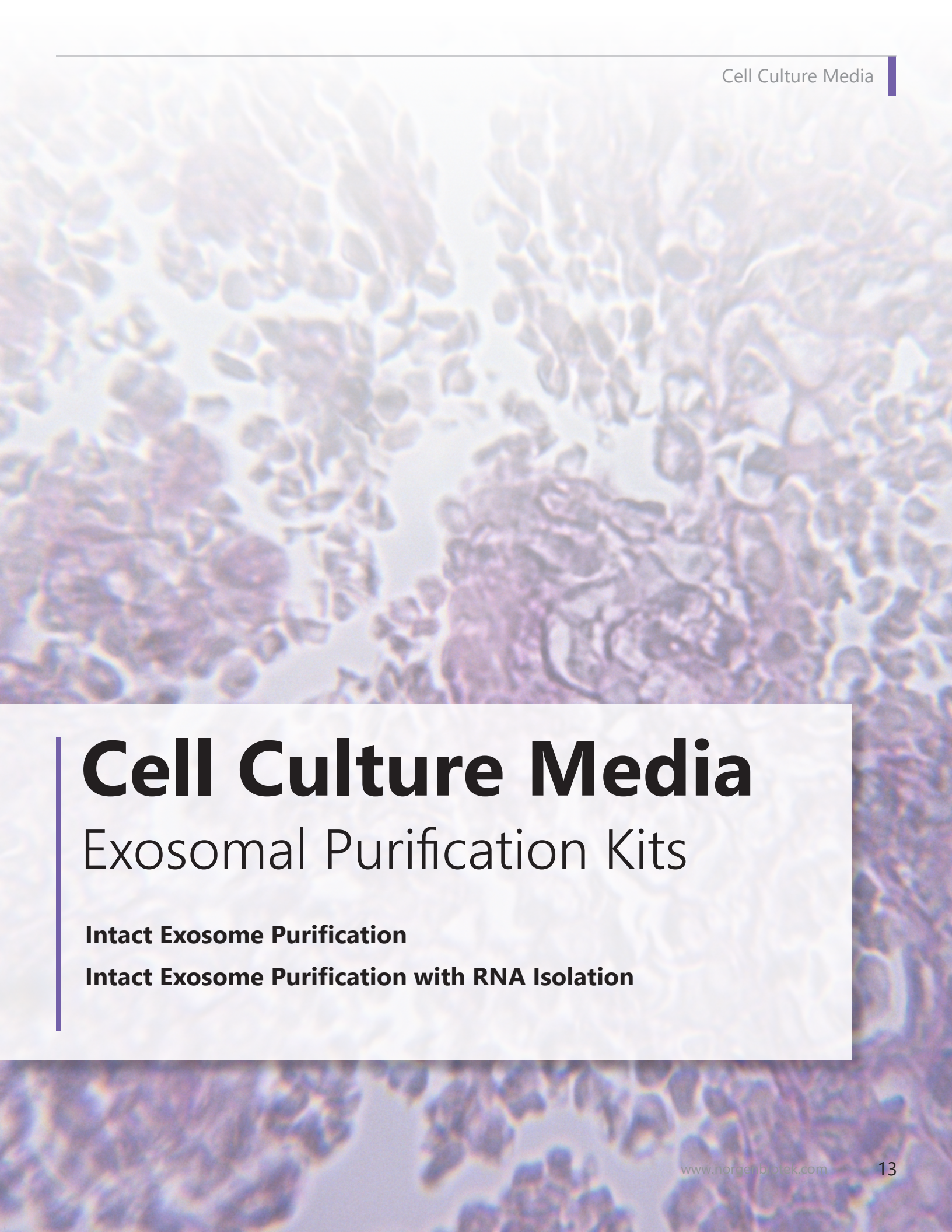

Cell Culture Media

Exosomal Purification Kits

Intact Exosome Purification

Intact Exosome Purification with RNA Isolation

Cell Culture Media Exosome Purification Kits

Cat No. 60400, 60500, 60600

- No time-consuming ultracentrifugation, filtration or special syringes required
- No precipitation reagents or overnight incubation required
- No protease treatment required
- Compatible with cell culture media from any species
- Pure exosomes are purified and are free-from any other RNA-binding proteins
- Purified exosomes can be analyzed using NanoSight® or Electron Microscopy for assessing the approximate exosome size range and concentration

Purification and enrichment of **intact cell culture media exosomes** for functional studies

Norgen's Cell Culture Media Exosome Purification Kits constitute an all-in-one system for the purification of cell culture media exosomes from different media volumes ranging from 5 mL to 35 mL. These kits also allow for the purification of intact extracellular vesicles (EVs) from cell culture media, and these EVs are ready for any downstream application. The purification is based on Norgen's proprietary resin. These kits provide a clear advantage over other available kits in that they do not require any special instrumentation, precipitation reagents or any protease treatments. More importantly, the purified exosomes will not be contaminated with any other RNA-bound proteins that may contaminate your exosomal RNA, which is essential if studying Exosomal RNA gene expression.

Versatile sample input ranging from 5 mL - 35 mL

Feature	Specification
Mini Kit Input Range	5 mL - 10 mL
Midi Kit Input Range	10 mL - 20 mL
Maxi Kit Input Range	20 mL - 35 mL
Size of Exosomes Purified	40 nm - 150 nm
Time to Complete 10 Purifications	15 - 30 minutes

Ordering Information

Description	Size	Cat. Number
Cell Culture Media Exosome Purification Mini Kit	50 preps	60400
Cell Culture Media Exosome Purification Midi Kit	25 preps	60500
Cell Culture Media Exosome Purification Maxi Kit	15 preps	60600

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Mini Kit

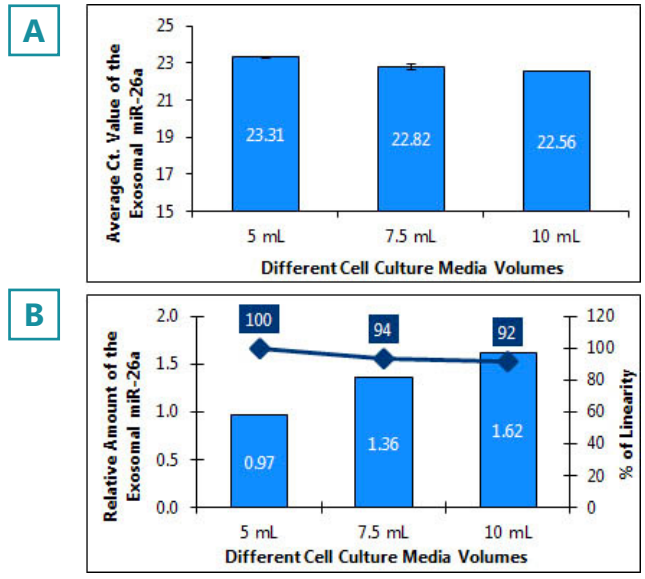

Figure 1. Isolation of RNA from exosomes purified from different cell culture media volumes. Norgen's Cell Culture Media Exosome Purification Mini Kit (Cat# 60400) was used to isolate exosomal RNA from exosomes purified from different cell culture media volumes using the same kit. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated exosomal RNA (A) The exosomal miR-26a is linearly decreasing with increasing the sample input volume. (B) The relative amount of the exosomal miR-26a shows excellent linearity with a percentage of recovery of more than 90%.

Midi Kit

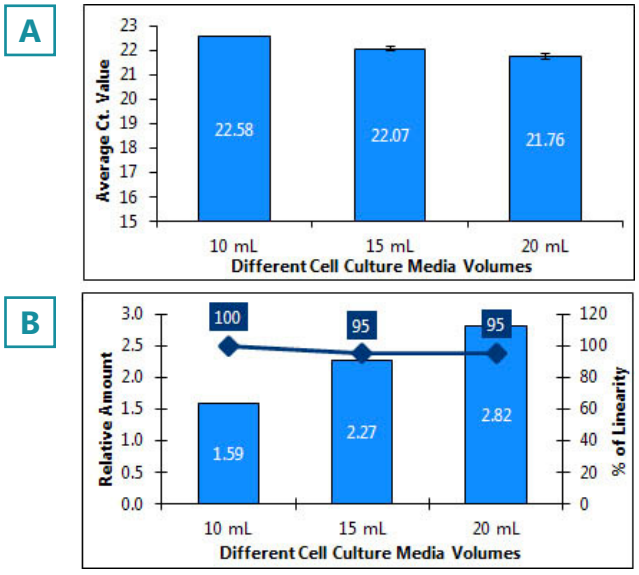

Figure 2. Isolation of RNA from exosomes purified from different cell culture media volumes. Norgen's Cell Culture Media Exosome Purification Midi Kit (Cat# 60500) was used to isolate exosomal RNA from exosomes purified from different cell culture media volumes using the same kit. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolatedexosomal RNA. (A) The exosomal miR-26a is linearly decreasing with increasing the sample input volume. (B) The relative amount of the exosomal miR-26a shows excellent linearity with a percentage of recovery of more than 90%.

Maxi Kit

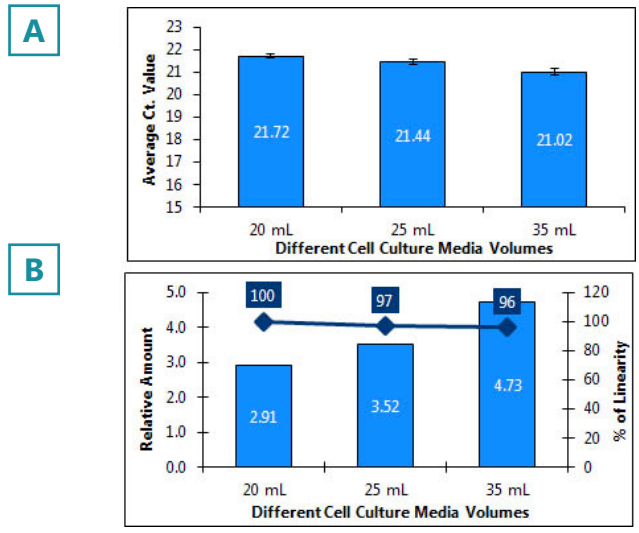

Figure 3. Isolation of RNA from exosomes purified from different cell culture media volumes. Norgen's Cell Culture Media Exosome Purification Maxi Kit (Cat# 60600) was used to isolate exosomal RNA from different cell culture media volumes from exosomes purified using the same kit. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated exosomal RNA. (A) The exosomal miR-26a is linearly decreasing with increasing the sample input volume. (B) The relative amount of the exosomal miR-26a shows excellent linearity with a percentage of recovery of more than 90%.

Cell Culture Media Exosome Purification and RNA Isolation Kits

Cat No. 60700, 60800, 60900

- Purification and enrichment of intact cell culture media exosomes for functional studies
- Isolate all sizes of exosomal and extracellular vesicle RNA, including microRNA
- Bind and elute all RNA irrespective of size or GC content, without bias
- No phenol extractions, Proteinase K treatment, or carrier RNA required
- No time-consuming ultracentrifugation, filtration nor special syringes required
- No precipitation reagents nor overnight incubation required
- Pure exosomes are purified and are free-from any other RNA-binding proteins

Rapid and simple purification of **intact exosomes and RNA isolation** from urine samples

Norgen's Cell Culture Media Exosome Purification and RNA Isolation Kits constitute an all-in-one system for the purification of exosomes and the subsequent isolation of exosomal RNA from different cell culture media sample volumes ranging from 5 mL to 35 mL. The purification is based on spin-column chromatography that employs Norgen's proprietary resin. The kit is designed to isolate all sizes of extracellular vesicle RNA, including microRNA. The kit provides a clear advantage over other available kits in that it does not require any special instrumentation, protein precipitation reagents, extension tubes, phenol/chloroform or protease treatments. Moreover, the kit allows the user to elute into a flexible elution volume ranging from 50 µL to 100 µL. The purified RNA is free from any protein-bound circulating RNA and of the highest integrity. The purified RNA can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Versatile sample input ranging from 5 mL - 35 mL

Feature	Specification
Mini Kit Input Range	5 mL - 10 mL
Midi Kit Input Range	10 mL - 20 mL
Maxi Kit Input Range	20 mL - 35 mL
Size of Exosomes Purified	40 nm - 150 nm
Time to Complete 10 Purifications	15 - 45 minutes

Ordering Information

Description	Size	Cat. Number
Cell Culture Media Exosome Purification and RNA Isolation Mini Kit	50 preps	60700
Cell Culture Media Exosome Purification and RNA Isolation Midi Kit	25 preps	60800
Cell Culture Media Exosome Purification and RNA Isolation Maxi Kit	15 preps	60900

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

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Mini Kit

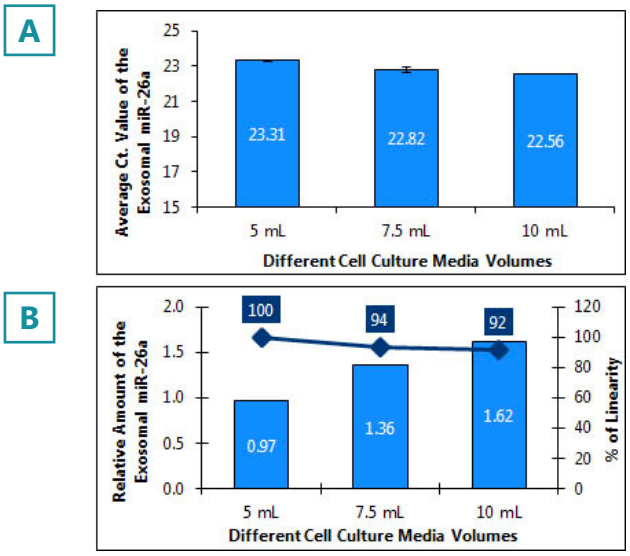

Figure 1. Isolation of RNA from exosomes purified from different cell culture media volumes. Norgen's Cell Culture Media Exosome Purification and RNA Isolation Mini Kit (Cat# 60700) was used to isolate exosomal RNA from exosomes purified from different cell culture media volumes using the same kit. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated exosomal RNA (A) The exosomal miR-26a is linearly decreasing with increasing the sample input volume. (B) The relative amount of the exosomal miR-26a shows excellent linearity with a percentage of recovery of more than 90%.

Midi Kit

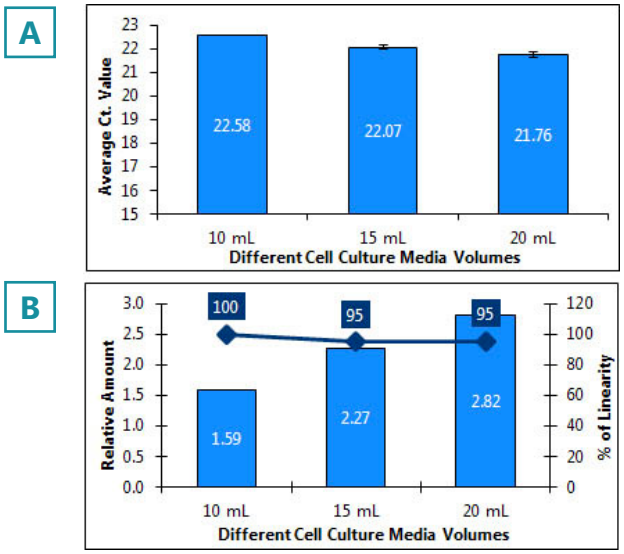

Figure 2. Isolation of RNA from exosomes purified from different cell culture media volumes. Norgen's Cell Culture Media Exosome Purification and RNA Isolation Midi Kit (Cat# 60800) was used to isolate exosomal RNA from exosomes purified from different cell culture media volumes using the same kit. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolatedexosomal RNA. (A) The exosomal miR-26a is linearly decreasing with increasing the sample input volume. (B) The relative amount of the exosomal miR-26a shows excellent linearity with a percentage of recovery of more than 90%.

Maxi Kit

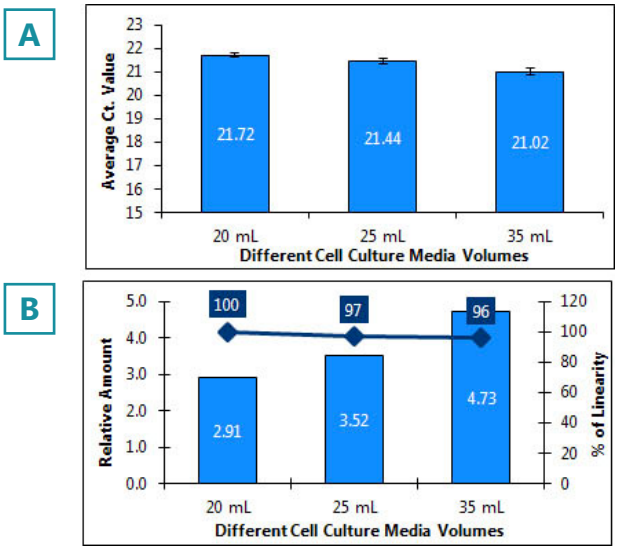

Figure 1. Isolation of RNA from exosomes purified from different cell culture media volumes. Norgen's Cell Culture Media Exosome Purification and RNA Isolation Maxi Kit (Cat# 60900) was used to isolate exosomal RNA from different cell culture media volumes from exosomes purified using the same kit. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated exosomal RNA. (A) The exosomal miR-26a is linearly decreasing with increasing the sample input volume. (B) The relative amount of the exosomal miR-26a shows excellent linearity with a percentage of recovery of more than 90%.

Select Publication

Using Norgen's Urine Exosome Purification and RNA Isolation Kit

Cancer cells exploit an orphan RNA to drive metastatic progression

Lisa Fish, Steven Zhang, Johnny X. Yu, Bruce Culbertson, Alicia Y. Zhou, Andrei Goga & Hani Goodarzi;

Nature Medicine 24, 1743–1751 (2018)

Abstract

Here we performed a systematic search to identify breast-cancer-specific small noncoding RNAs, which we have collectively termed orphan noncoding RNAs (oncRNAs). We subsequently discovered that one of these oncRNAs, which originates from the 3′ end of TERC, acts as a regulator of gene expression and is a robust promoter of breast cancer metastasis. This oncRNA, which we have named T3p, exerts its prometastatic effects by acting as an inhibitor of RISC complex activity and increasing the expression of the prometastatic genes NUPR1 and PANX2. Furthermore, we have shown that oncRNAs are present in cancer-cell-derived extracellular vesicles, raising the possibility that these circulating oncRNAs may also have a role in non–cell autonomous disease pathogenesis. Additionally, these circulating oncRNAs present a novel avenue for cancer fingerprinting using liquid biopsies.

Select Methods

Extracellular-vesicle RNA was isolated from 5ml conditioned medium, prepared as outlined above, using the Cell Culture Media Exosome Purification and RNA Isolation kit (Norgen Biotek)[...] Total cellular smRNA samples were extracted using the Norgen Biotek Small RNA Purification kit according to the manufacturer's protocol.

Extracellular-vesicle RNA was isolated from serum samples using the Plasma/ Serum Exosome Purification and RNA isolation kit (Norgen Biotek) according tothe manufacturer's instructions. [...] In addition, we also performed qPCR assays. For this, we extracted smRNAs from MDA-MB-231 parental cells and their highly metastatic MDA-LM2 derivative cell line (microRNA Purification Kit, Norgen)

(Fish *et al.* 2018, p. 10)

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www.nature.com

Urine

Exosomal Purification Kits

Intact Exosome Purification

Intact Exosome Purification with RNA Isolation

Exosomal and Free-Circulating RNA Fractionation

Urine Exosome Purification Kits

Cat No. 57700, 57800, 57900

- Purification and enrichment of intact urinary exosomes for functional studies
- No time-consuming ultracentrifugation, filtration nor special syringes required
- No precipitation reagents nor overnight incubation required
- No protease treatment required
- Compatible with urine from any species
- Pure exosomes are purified and are free-from any other RNA-binding proteins
- Purified exosomes can be analyzed using NanoSight® or Electron Microscopy for assessing the approximate exosome size range and concentration

Rapid and simple purification of **intact exosomes** from urine samples

Norgen's Urine Exosome Purification Kits constitute an all-in-one system for the purification of urinary exosomes from different urine sample volumes ranging from 250 µL to 30 mL. These kits also allow for the purification of intact extracellular vesicles (EVs) from different urine sample volumes, and these EVs are ready for any downstream application. The purification is based on Norgen's proprietary resin. These kits provide a clear advantage over other available kits in that they do not require any special instrumentation, precipitation reagents or any protease treatments. More importantly, the purified exosomes will not be contaminated with any other RNA-bound proteins that may contaminate your exosomal RNA, which is essential if studying Exosomal RNA gene expression.

Versatile sample input ranging from 250 µL - 30 mL

Feature	Specification
Mini Kit Input Range	250 µL - 1 mL
Midi Kit Input Range	2 mL - 10 mL
Maxi Kit Input Range	11 mL - 30 mL
Size of Exosomes Purified	40 nm - 150 nm
Time to Complete 10 Purifications	15 - 30 minutes

Ordering Information

Description	Size	Cat. Number
Urine Exosome Purification Mini Kit	50 preps	57700
Urine Exosome Purification Midi Kit	25 preps	57800
Urine Exosome Purification Maxi Kit	15 preps	57900

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
SCAN HERE

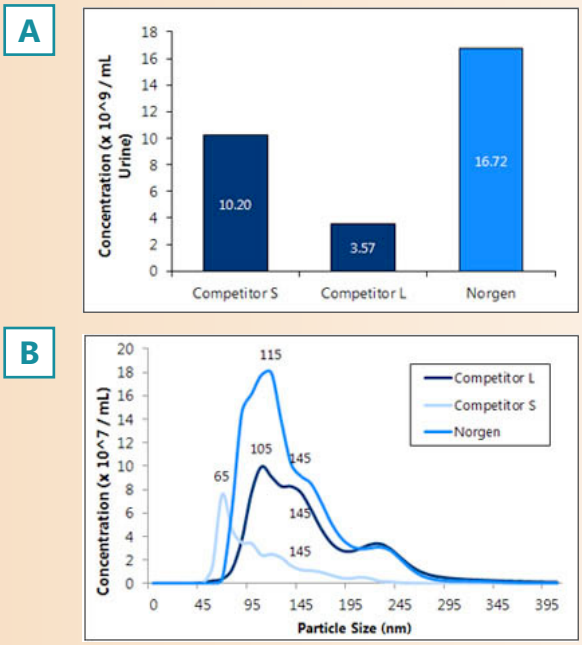

Figure 1. Intact exosomes purified from 5 mL using different purification methods. Intact exosomes were purified from 5 mL urine using Norgen's Urine Exosome Purification Midi Kit (Cat# 57800), Competitor S's kit, and Competitor L's kit. Exosomes purified using Norgen's kit were resuspended in 400 µL Norgen's ExoR buffer, diluted 1:1,000 and visualized on the NanoSight LM10 instrument. The analysis shows that Norgen's kit isolated 115 nm exosomes with a recovery of 8.36 x 109 particles/mL urine samples. No impurities were found to be contaminating the exosomes purified using Norgen's Urine Exosome Purification Midi Kit. Additionally, Exosomes with a broader size range covering from 75nm - 250nm were purified from 5 mL urine with a higher concentration as compared to the other two methods.

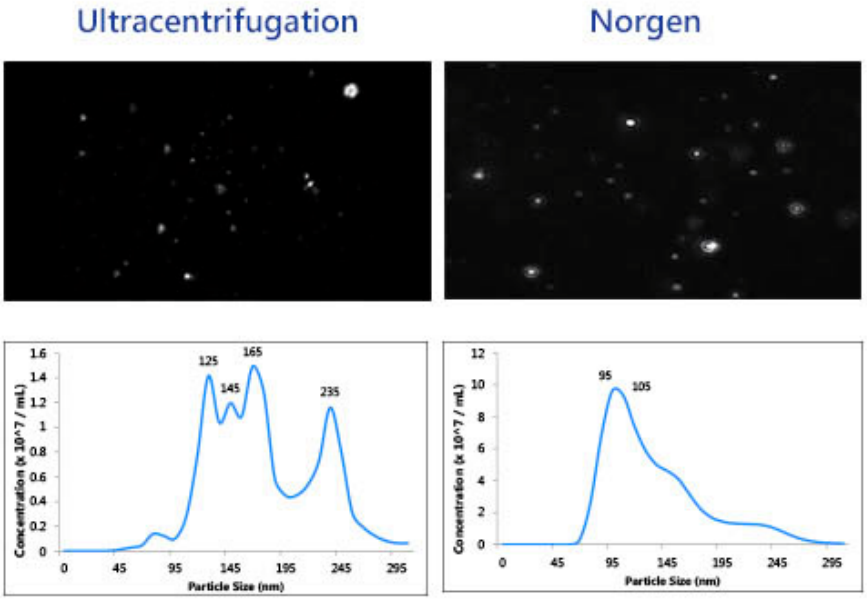

Figure 2. Intact Exosomes were purified from 10 mL urine using Norgen's Urine Exosome Purification Midi Kit (Cat# 57800) and Ultracentrifugation. Exosomes purified using Norgen's kit and ultracentrifugation were resuspended in 400 µL of Norgen's ExoR buffer, diluted 1:1,000 and visualized on the NanoSight LM10 instrument. The analysis shows that Norgen's kit purified exosomes with sizes ranging from 65 nm to 195 nm, with a total recovery of 7.63 x 108 particles/mL. No impurities were found to be contaminating the exosomes purified using Norgen's Urine Exosome Purification Midi Kit as opposed to the exosomes purified using ultracentrifugation, which purified exosomes with larger particle sizes ranging from 125 nm - 235 nm with a total recovery of 1.56 x 108 particles/mL.

Urine Exosome Purification and RNA Isolation Kits

Cat No. 58400, 58700, 58800

- Purification and enrichment of intact urinary exosomes for functional studies
- Bind and elute all RNA irrespective of size or GC content, without bias
- Isolate all sizes of exosomal and extracellular vesicle RNA, including microRNA
- No phenol extractions, Proteinase K treatment, nor carrier RNA required
- No time-consuming ultracentrifugation, filtration nor special syringes are required
- No precipitation reagents nor overnight incubation required
- Compatible with urine from any species
- Pure exosomes are purified and are free-from any other RNA-binding proteins

Rapid and simple purification of **intact exosomes and RNA isolation** from urine samples

Norgen's Urine Exosome Purification and RNA Isolation Kits constitute an all-in-one system for the purification of exosomes and the subsequent isolation of exosomal RNA from different urine sample volumes ranging from 250 µL to 30 mL. The purification is based on spin-column chromatography that employs Norgen's proprietary resin. The kit is designed to isolate all sizes of extracellular vesicle RNA, including microRNA. The kit provides a clear advantage over other available kits in that it does not require any special instrumentation, protein precipitation reagents, extension tubes, phenol/chloroform or protease treatments. Moreover, the kit allows the user to elute into a flexible elution volume ranging from 50 µL to 100 µL. The purified RNA is free from any protein-bound circulating RNA and of the highest integrity. The purified RNA can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Versatile sample input ranging from 250 µL - 30 mL

Feature	Specification
Mini Kit Input Range	250 µL - 1 mL
Midi Kit Input Range	2 mL - 10 mL
Maxi Kit Input Range	11 mL - 30 mL
Size of Exosomes Purified	40 nm - 150 nm
Time to Complete 10 Purifications	15 - 40 minutes

Ordering Information

Description	Size	Cat. Number
Urine Exosome Purification and RNA Isolation Mini Kit	50 preps	58400
Urine Exosome Purification and RNA Isolation Midi Kit	25 preps	58700
Urine Exosome Purification and RNA Isolation Maxi Kit	15 preps	58800

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
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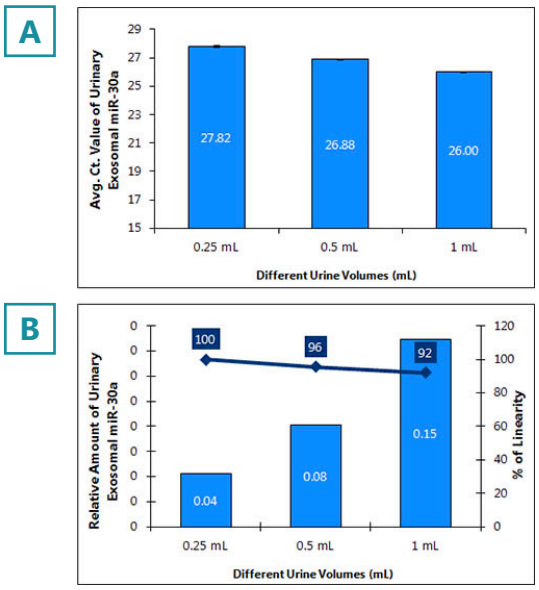

Figure 1. Isolation of RNA from exosomes purified from different urine volumes. Norgen's Urine Exosome Purification and RNA Isolation Mini Kit (Cat# 58400) was used to isolate RNA from exosomes purified from different urine volumes using the same kit. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated urinary exosomal miR-30a. (A) The urinary exosomal miR-30a is linearly decreasing with increasing the sample input volume. B) The relative amount of the urinary exosomal miR-30a shows excellent linearity with a percentage of recovery of more than 90%.

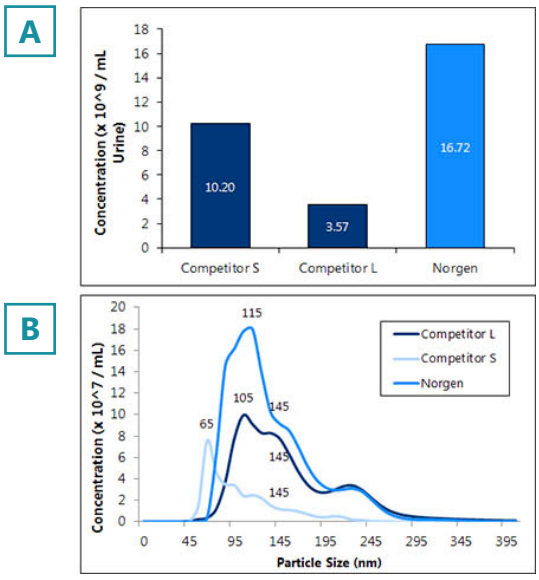

Figure 2. Intact exosomes purified from 5 mL of urine using different purification methods. Intact exosomes were purified from 5 mL urine using Norgen's Urine Exosome Purification and RNA Isolation Midi Kit (Cat# 58700), Competitor S's kit, and Competitor L's kit. Exosomes purified using Norgen's kit were resuspended in 400 µL Norgen's ExoR buffer, diluted 1:1,000 and visualized on the NanoSight LM10 instrument. The analysis shows that Norgens kit isolated 115 nm exosomes with a recovery of 8.36 x 10^9 particles/mL urine samples. No impurities were found to be contaminating the exosomes purified using Norgen's Urine Exosome Purification and RNA Isolation Midi kit. Additionally, exosomes with a broader size range covering from 75nm to 250nm were purified from 5 mL urine with a higher concentration as compared to the other two methods.

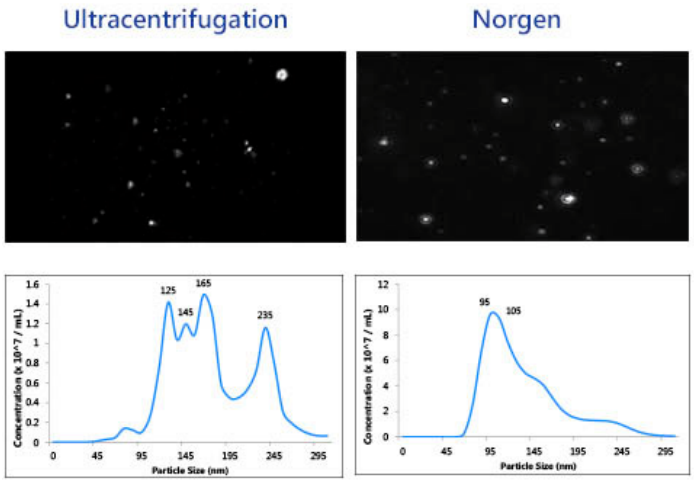

Figure 3. Intact Exosomes were purified from 10 mL urine using Norgen's Urine Exosome Purification and RNA Isolation Midi Kit (Cat# 58700) and ultracentrifugation. Exosomes purified using Norgens kit and ultracentrifugation was resuspended in 400 µL of Norgens ExoR buffer, diluted 1:1,000 and visualized on the NanoSight LM10 instrument. The analysis shows that Norgen's kit purified exosomes with sizes ranging from 65 nm - 195 nm, with a total recovery of 7.63 x 108 particles/mL. No impurities were found to be contaminating the exosomes purified using Norgen's Urine Exosome Purification and RNA Isolation Midi Kit as opposed to the exosomes purified using ultracentrifugation, which purified exosomes with larger particle sizes ranging from 125 nm to 235 nm with a total recovery of 1.56 x 108 particles/mL.

Urine Exosome and Free-Circulating RNA Fractionation Kits

Cat No. 59200, 59300, 59400

- Isolate all sizes of exosomal and extracellular vesicle RNA, including microRNA
- Versatile sample input ranging from 250 µL to 30 mL
  Isolate all sizes of free-circulating RNA, including microRNA
- Bind and elute all RNA irrespective of size or GC content, without bias
- The purified exosomal RNA is free from any circulating RNA-binding proteins
- No phenol extractions, Proteinase K treatment nor carrier RNA required
- No time-consuming ultracentrifugation, filtration nor special syringes are required
- No precipitation reagents or overnight incubation required
- Concentrate isolated exosomal RNA and free-circulating RNA into a flexible elution volume ranging from 50 µL to 100 µL
- Purify high-quality RNA in 15-20 minutes

Rapid and simple sequential isolation of **exosomal** and **free-circulating RNA** from different urine sample volumes

Norgen's Urine Exosome and Free-Circulating RNA Isolation Kits constitute an all-in-one system for the purification of exosomes and the subsequent isolation of exosomal RNA from different urine sample volumes ranging from 250 µL to 30 mL. The purification is based on spin-column chromatography that employs Norgen's proprietary resin. The kit is designed to isolate all sizes of extracellular vesicle RNA, including microRNA. The kit provides a clear advantage over other available kits in that it does not require any special instrumentation, protein precipitation reagents, extension tubes, phenol/chloroform or protease treatments. Moreover, the kit allows the user to elute into a flexible elution volume ranging from 50 µL to 100 µL. The RNA isolated from the purified exosomes is free from any protein-bound circulating RNA and is of the highest integrity. Moreover, the free-circulating, protein-bound, RNA is free from any exosomal RNA. The purified RNA can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays

Versatile sample input ranging from 250 µL - 30 mL

Feature	Specification
Mini Kit Input Range	250 µL - 1 mL
Midi Kit Input Range	2 mL - 10 mL
Maxi Kit Input Range	11 mL - 30 mL
Size of Exosomes Purified	40 nm - 150 nm
Time to Complete 10 Purifications	15 - 40 minutes

Ordering Information

Description	Size	Cat. Number
Urine Exosome and Free-Circulating RNA Isolation Mini Kit	50 preps	59200
Urine Exosome and Free-Circulating RNA Isolation Midi Kit	25 preps	59300
Urine Exosome and Free-Circulating RNA Isolation Maxi Kit	15 preps	59400

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
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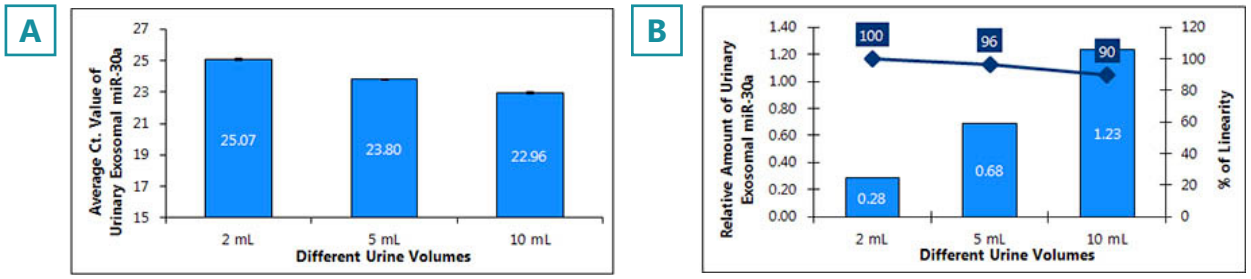

Figure 1. Isolation of RNA from exosomes purified from different urine volumes. Norgen's Urine Exosome and Free-Circulating RNA Isolation Midi Kit (Cat# 59300) was used to isolate Exosomal RNA from different urine volumes ranging from 2 mL and up to 10 mL. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated urinary exosomal miR-30a. (A) The urinary exosomal miR-30a is linearly decreasing with increasing the sample input volume. B) The relative amount of the urinary exosomal miR-30a shows excellent linearity with a percentage of recovery of more than 90%.

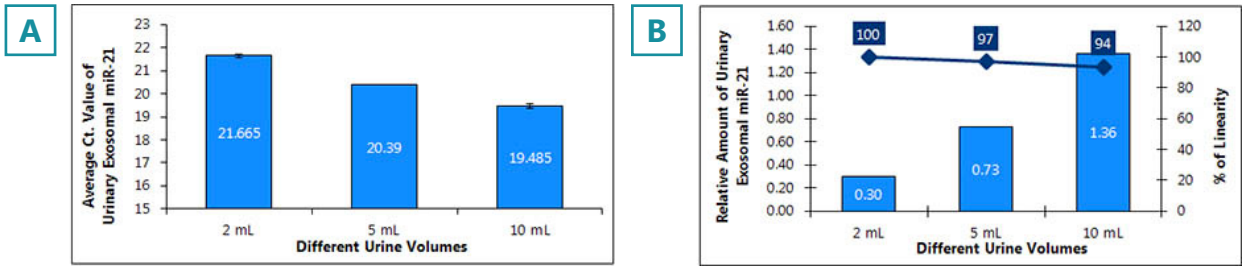

Figure 2. Isolation of Free-Circulating RNA after the isolation of Exosomal RNA from different urine volumes. Norgen's Urine Exosome and Free-Circulating RNA Isolation Midi Kit (Cat# 59300) was used to isolate Free-Circulating RNA from after the isolation of Exosomal RNA from different urine volumes ranging from 2 mL and up to 10 mL. Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated urinary free-circulating miR-21. (A) The free-circulating miR-21 is linearly decreasing with increasing the sample input volume. B) The relative amount of the free-circulating miR-21 shows excellent linearity with a percentage of recovery of more than 90%.

Urine Exosome RNA Isolation Kit

Cat No. 47200

- Isolation of exosomal RNA molecules from urine samples
- Rapid and convenient spin-column protocol
- Isolate inhibitor-free urinary microRNA for any application
- Purification of exosomal proteins for western blot analysis
- Purified exosomes can be analyzed using NanoSight® or Electron Microscopy for assessing the approximate exosome size range and concentration
- Purified RNA is suitable for a variety of downstream applications, including Small RNA Sequencing. Find out more information on Norgen's NGS services

A rapid procedure for the isolation of exosomal RNA from urine samples

This kit provides a rapid spin-column procedure for the isolation of exosomal RNA from urine samples. Users can simultaneously concentrate and isolate high quality exosomal RNA, including microRNA, for use in sensitive downstream assays such as RT-PCR, qRT-PCR, NGS, microarrays and more. The protocol can be completed in under 30 minutes. Urine volumes of 1 to 10 mL can be processed easily and rapidly. All sizes of RNA are recovered at an equal rate without the need for any phenol steps.

Versatile sample input ranging from 250 µL - 30 mL

Feature	Specification
Minimum Urine Input	1 mL
Maximum Urine Input	10 mL
Size of RNA Purified	Small exosomal RNA species
Time to Complete 10 Purifications	~ 50 minutes

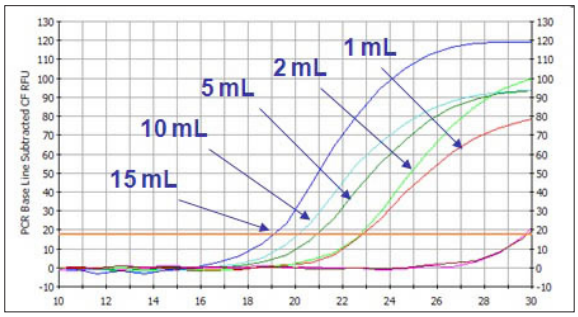

Figure 1. Detection of Urine Exosomal RNA Isolated from Different Urine Volumes. Norgen's Urine Exosome RNA Isolation Kit was used to isolate urine exosomal RNA from different urine volumes ranging from 1 to 15 mL. The purified urine exosomal RNA was then used as the template in an RT-qPCR reaction to detect the human 5S gene. Three microlitres of the isolated urine exosomal RNA was used as the template in the RT step, and 3 µL from the RT step was used in the qPCR reaction. As it can be seen, the qPCR was able to successfully detect and amplify the 5S gene from RNA isolated from different urine volumes, indicating the high quality of the isolated urine exosomal RNA.

Ordering Information

Description	Size	Cat. Number
Urine Exosome RNA Isolation Kit	50 preps	47200

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
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NGS on Illumina MiSeq

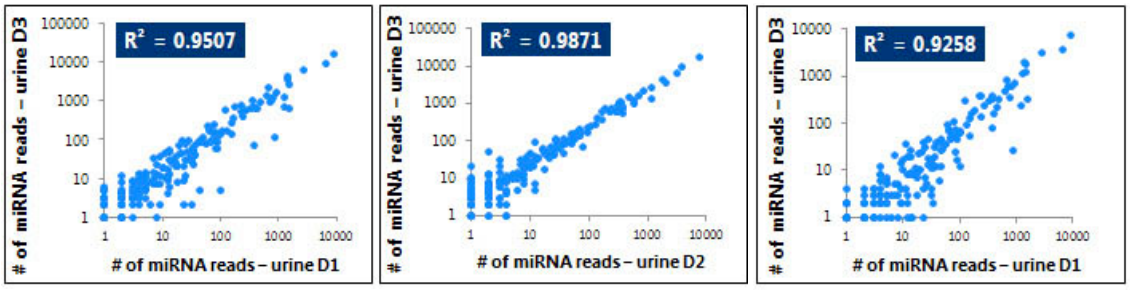


Figure 2. High Diversity and Consistency of miRNA Recovered from Urine. Mid-stream urine was collected from three different healthy individuals. RNA was isolated from 20 mL of each sample using Norgen's Urine Exosomal RNA Purification Kit (Cat# 47200). Small RNA Libraries were then generated using an Illumina TruSeq Small RNA Library Preparation Kit and subsequently sequenced on an Illumina MiSeq system. The normalized read counts of the mapped miRNAs were then compared between each individual sample. The scatter plots showed that the miRNA diversity was highly conserved among all individuals tested (R2 ≥ 0.92). This suggests that Norgen's RNA purification workflow recovers consistent profiles of small RNAs. This could enhance the possibility of detecting potential urine-based small RNA biomarkers.

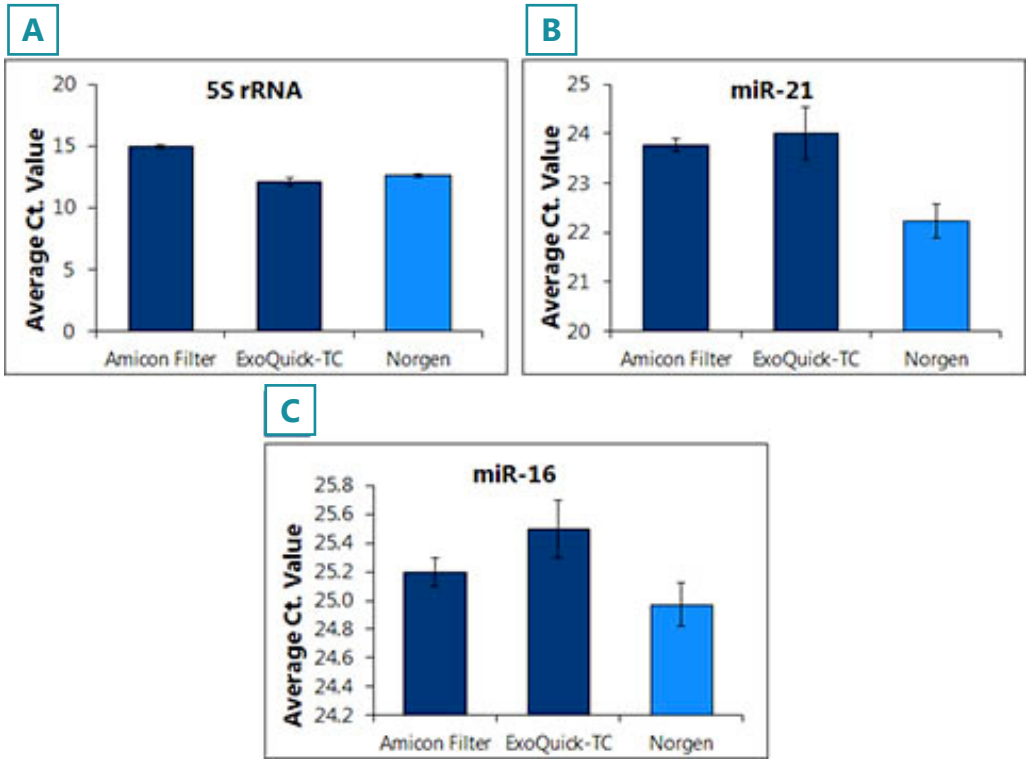


Figure 3. Effective and Consistent Detection of Urine Exosome RNA. Norgen's Urine Exosome RNA Isolation Kit can effectively isolate RNA from urine. Urine exosome RNA was isolated from 5 mL of human cell-free urine using Norgen's Urine Exosome RNA Isolation Kit (blue), ExoQuick-TC Exosome Precipitation Reagent (green) and Amicon® Ultra-15 Filter (red). Stem loop RT-qPCR using primers specific to miR-21 and miR-16 as well as the housekeeping 5S rRNA was performed. In brief, three microliters of the 100 µL isolated RNA was then subjected to a 20 µL reverse transcription using 5S rRNA, miR-21 and miR-16 stem-loop reverse primer or reveres primer. Three microliters of the reverse transcription was used in a 20 µL real-time PCR reaction with primers to detect the human miR-21, human miR-16 and the 5S rRNA. Norgen's Urine Exosome RNA Isolation Kit is the only product that showed consistent detection of all tested transcripts with the highest quality.

Select Publication Using Norgen's Urine Exosome Purification Kit

Evaluation of optimal extracellular vesicle small RNA isolation and qRT-PCR normalisation for serum and urine.

Crossland RE[1], Norden J[2], Bibby LA[2], Davis J[2], Dickinson AM[2].

[1]Institute of Cellular Medicine, Medical School, Framlington Place, Newcastle University, Newcastle upon Tyne NE2 4HH, UK. Electronic address: rachel.crossland@ncl.ac.uk.
[2]Institute of Cellular Medicine, Medical School, Framlington Place, Newcastle University, Newcastle upon Tyne NE2 4HH, UK.

J Immunol Methods. 2016 Feb;429:39-49. doi: 10.1016/j.jim.2015.12.011. Epub 2015 Dec 23.

Abstract

MicroRNAs are small regulatory molecules that demonstrate useful biomarker potential. They have been recognised in biofluids, where they are protected from degradation by encapsulation into extracellular vesicles (EVs). A number of commercial products are available for the isolation of EVs and their RNA content; however, extensive protocol comparisons are lacking. Furthermore, robust qRT-PCR assessment of microRNA expression within EVs is problematic, as endogenous controls (ECs) previously used in cellular samples may not be present. This study compares EV isolation and RNA extraction methods (EV precipitation reagents, RNA isolation kits and ultracentrifugation) from serum or urine samples and evaluates suitable ECs for incorporation into qRT-PCR analysis. Results were assessed by electron microscopy, nanoparticle tracking analysis and bioanalyzer concentrations. The stability of 8 ECs was compared for both serum and urine EV RNA and retrospectively validated in independent cohorts (serum n=55, urine n=50). The Life Technologies precipitation reagent gave superior serum EV recovery compared to SBI reagent, as assessed by NTA size distribution, increased RNA concentration, and lower small RNA Ct values. Similarly, the Norgen Biotek Urine Exosome RNA Isolation Kit gave improved results for urine EV isolation compared to ultracentrifugation, when determined by the same parameters. The Qiagen miRNeasy™ RNA isolation kit gave suitable serum EV RNA concentrations compared to other kits, as assessed by Bioanalyzer and small RNA qRT-PCR. Small RNAs HY3 (S.D=1.77, CoV=6.2%) and U6 (S.D=2.14, CoV=8.6%) were selected as optimal ECs for serum EV microRNA expression analysis, while HY3 (S.D=1.67, CoV=6.5%) and RNU48 (S.D=1.85, CoV=5.3%) were identified as suitable for urine studies. In conclusion, this study identifies optimal methods for isolation of serum and urine EV RNA, and suitable ECs for normalisation of qRT-PCR studies. Such reports should aid in the standardisation of EV microRNA data, particularly for biomarker studies.

For the full access to this publication please visit

www.pubmed.gov

Select Publication

Using Norgen's Urine Exosome Purification and RNA Isolation Kit

miRNA profiling of urinary exosomes to assess the progression of acute kidney injury

Hiroko Sonoda, Byung Rho Lee, Ki-Hoon Park, Deepak Nihalani, Je-Hyun Yoon, Masahiro Ikeda & Sang-Ho Kwon;

Scientific Reports 9, Article number: 4692 (2019)

Abstract

Because exosomes have gained attention as a source of biomarkers, we investigated if miRNAs in exosomes (exo-miRs) can report the disease progression of organ injury. Using rat renal ischemia-reperfusion injury (IRI) as a model of acute kidney injury (AKI), we determined temporally-released exo-miRs in urine during IRI and found that these exo-miRs could reliably mirror the progression of AKI. From the longitudinal measurements of miRNA expression in kidney and urine, we found that release of exo- miRs was a regulated sorting process. In the injury state, miR-16, miR-24, and miR-200c were increased in the urine. Interestingly, expression of target mRNAs of these exo-miRs was significantly altered in renal medulla. Next, in the early recovery state, exo-miRs (miR-9a, miR-141, miR-200a, miR-200c, miR-429), which share Zeb1/2 as a common target mRNA, were upregulated together, indicating that they reflect TGF-β-associated renal fibrosis. Finally, release of exo-miRs (miR-125a, miR-351) was regulated by TGF-β1 and was able to differentiate the sham and IRI even after the injured kidneys were recovered. Altogether, these data indicate that exo-miRs released in renal IRI are associated with TGF-β signaling. Temporal release of exo-miRs which share targets might be a regulatory mechanism to control the progression of AKI.

RNA extraction from exosomes, kidneys, and cell lines.
6-hour urine collection was done at the time points shown in Fig. 1a, and collected urine was immediately stored at −80°C until RNA extraction. Frozen urine samples, each with a volume of 1 ml, were thawed on rotator for 15 min and then centrifuged at 17,000 x g for 10min. Thereafter, small RNAs in urine were extracted, using the Urine exosome purifcation and RNA isolation kit (Norgen Biotek) according to the manufacturer's instructions.

(Sonoda *et al.* 2019, p. 2)

For the full access to this publication please visit

www.nature.com

Saliva

Exosomal Purification Kits

Intact Exosome Purification

Intact Exosome Purification with RNA Isolation

Exosomal and Free-Circulating RNA Fractionation

Saliva Exosome Collection and Preservation Kit

Cat No. 65400

- Reliable and cost-effective
- Non-invasive, user-friendly sample collection
- Samples are non-infectious and can be handled and shipped safely
- Preserved exosomes are stable for 2 years at ambient temperature
- Shipping accessories can be purchased separately Compatible with **Norgen's Saliva Exosome Purification Kit (Cat# 65300)**
- RNA can be isolated from the purified exosomes using **Norgen's Exosome RNA Purification kit (Cat# 58000)**

Sample collection and preservation of Exosomes in one convenient kit

Norgen's Saliva Exosome Collection and Preservation Kit is an all-in-one solution designed for 1) simple and non-invasive saliva collection; 2) preservation of exosomes in saliva samples at ambient temperature; and 3) isolation of high quality intact exosomes and exosomal RNA within a laboratory setting. The Saliva Exosome Collection, and Preservation Kit contains 50 Individual Saliva Exosome Collection and Preservation Devices, as well as the required reagents for the subsequent laboratory purification of intact exosomes and the isolation of the exosomal RNA from the preserved samples. Each of the 50 individual Saliva Exosome Collection and Preservation Devices consists of 3 components: (1) Saliva Collection Funnel and Collection Tube that contains Norgen's Urine Preservative in a dried format, (2) Collection Tube Cap, and (3) ID Label. Saliva samples are collected by spitting inside the Collection Funnel which has been assembled with the Collection Tube. After collecting the required volume of saliva the Collection Funnel is removed, the Collection Tube is securely capped with Collection Tube Cap. The collected saliva is mixed by gentle inversion for several times till the saliva colour changes into yellow. The Saliva Collection Tube is subsequently sent to the laboratory for the purification of intact exosomes and for the subsequent isolation and analysis of saliva exosomal RNA. The saliva exosomes in preserved samples is stable for 2 years at room temperature.

Specifications

Feature	Specification
Volume of Saliva Collected	2 mL
Preservation Temperature	Room Temperature
Preservation Time	2 years

Ordering Information

Description	Size	Cat. Number
Saliva Exosome Collection and Preservation Kit	50 devices	65400

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
SCAN HERE

Saliva Exosome Purification Kit

Cat No. 65300

- Versatile sample input ranging from 0.5 mL to 2 mL
- Process fresh saliva or saliva collected with **Norgen's Saliva Exosome Collection and Preservation Kit (Cat# 65400).**
- No time-consuming ultracentrifugation, filtration nor special syringes required
- No precipitation reagents, overnight incubation required or protease treatment required
- Purify pure exosomes that are free from RNA-binding proteins
- Purified exosomes can be analyzed using NanoSight® or Electron Microscopy for assessing the approximate exosome size range and concentration
- RNA can be isolated from the purified exosomes using **Norgen's Exosomal RNA Isolation Kit (Cat# 58000)**

Purification and enrichment of **intact** saliva exosomes

Norgen's Saliva Exosome Purification Kit allows for the purification of saliva exosomes from 500 µL to 2 mL of fresh saliva samples or saliva samples collected using Norgen's Saliva Exosome Collection and Preservation Kit (Cat# 65400). This kit also allows for the purification of intact extracellular vesicles (EVs) from different saliva sample volumes, and these EVs are ready for any downstream application. The purification is based on Norgen's proprietary resin. These kits provide a clear advantage over other available kits in that they do not require any special instrumentation, precipitation reagents or any protease treatments. More importantly, the purified exosomes will not be contaminated with any other RNA-bound proteins that may contaminate your exosomal RNA, which is essential if studying exosomal RNA gene expression.

Versatile sample input ranging from 0.5 mL - 2 mL

Feature	Specification
Mini Column Input Range	0.5 mL - 2 mL
Size of Exosomes Purified	40 nm - 150 nm
Time to Complete 10 Purifications	15 - 30 minutes

Ordering Information

Description	Size	Cat. Number
Saliva Exosome Purification Kit	50 preps	65300

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
SCAN HERE

Exosome Depletion

FBS Depletion Kits

FBS Exosome Depletion Kit I & II (Column Format)

Cat No. 61200, 61300

- Deplete exosome-sized vesicles from versatile FBS volumes of up to 240 mL
- No protease treatment required
- No time-consuming ultracentrifugation
- No filtration or special syringes are required
- No precipitation reagents required
- No overnight incubation required
- Depleted FBS has no detectable cow's miRNA
- The depleted FBS provides the same cellular growth rates as the standard FBS

Efficient depletion of cow's exosomes from Fetal Bovine Serum

Norgen's FBS Exosome Depletion Kits (Column Format) constitute an all-in-one system for the depletion of cow's exosomes from FBS prior to using it as a growth supplement in your culture medium. The FBS recovered from the depletion process is exosome-depleted and does not contain any quantifiable bovine miRNAs. Moreover, the exosome-depleted FBS will support the growth of your cells of interest similar to the non-depleted FBS. Norgen's kits allows for the depletion of different FBS volumes with a maximum volume ranging from 120 mL to 240 mL. The depletion is based on Norgen's proprietary resin. These kits provide a clear advantage over other available kits in that they do not require ultracentrifugation, any special instrumentation, precipitation reagents or any protease treatments. More importantly, the depletion process is an inexpensive method for depletion of your own FBS, as compared to the current ready-to-use exosome-depleted media available on the market.

Versatile sample input up to 240 mL

Feature	Specification
Sample Type	Fetal Bovine Serum
Sample Volume Range	Up to 120 mL FBS Exosome Depletion Kit I (Slurry Format) Up to 240 mL FBS Exosome Depletion Kit II (Slurry Format)
Depletion	Deplete exosome-sized vesicle
Bovine miRNA	No detectable bovine miRNA
Time to Complete 6 Purifications	40 minutes

Ordering Information

Description	Size	Cat. Number
FBS Exosome Depletion Kit I (Column Format)	6 preps	61200
FBS Exosome Depletion Kit II (Column Format)	12 preps	61300

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
SCAN HERE

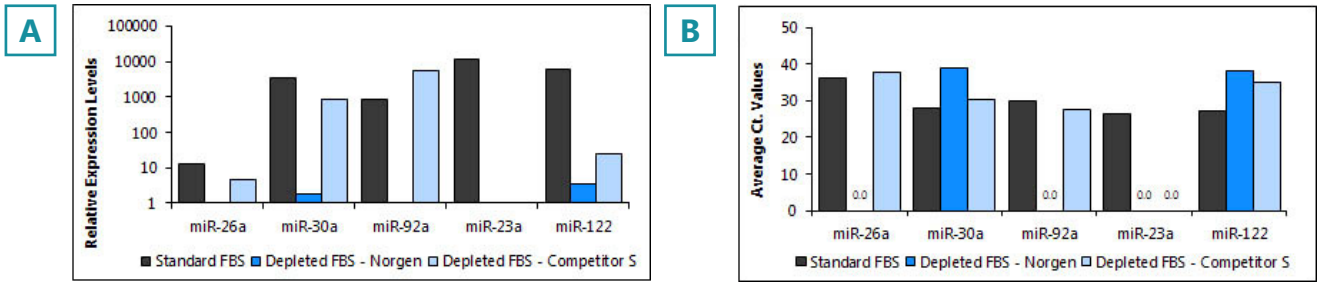


Figure 1. Exosome-depleted FBS with Norgen's FBS Exosome Depletion Kits (Column Format) has undetectable Bovine miRNA levels. Norgen's FBS Exosome Depletion Kit I (Column Format) (Cat# 61200) was used to deplete bovine miRNA from 5mL FBS. Total RNA/miRNA including exosomal RNA was purified from the depleted FBS, non-depleted FBS and a commercially available ready – to – go depleted FBS using Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Maxi Kit (Cat# 55800). Five different bovine microRNAs were assessed by RT-qPCR (miR-26a, miR-30a, miR-92a, miR-23a and miR-122). Three out of the five tested miRNA (miR-26a, miR-92a and miR-23a) didn't show any amplification in the FBS depleted using Norgen's FBS Exosome Depletion Kit I (Column Format) whereas the other two miRNAs (miR-30a and miR-122) showed very late Ct. values which appeared to be a primer dimer according to the melt curve.

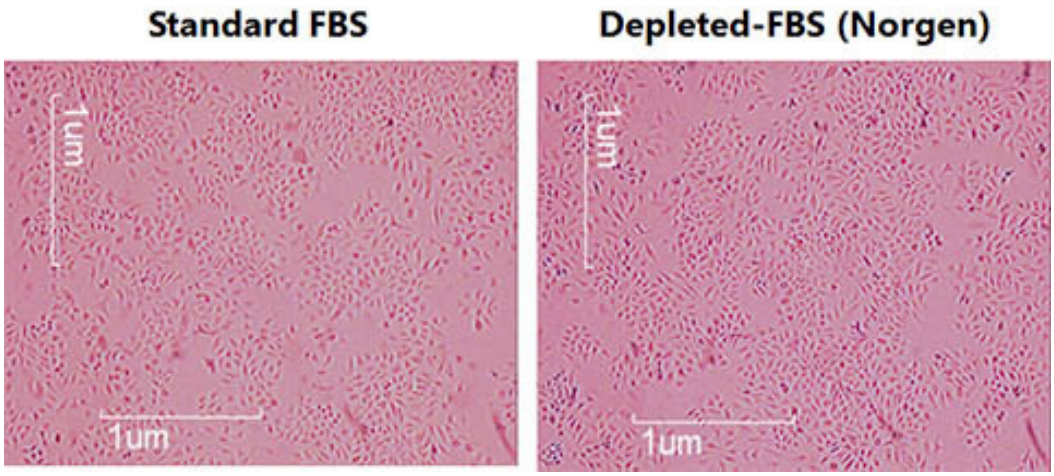


Figure 2. Growth rates of HeLa cells in media containing Exosome-depleted FBS. Growth rates of HeLa cells in media containing Exosome-depleted FBS using Norgen's FBS Exosome Depletion Kits (Column Format) was compared to that in media containing standard FBS. Simply, HeLa cells were seeded in DMEM with either 10% Exosome-depleted FBS using Norgen's Kits or 10% standard FBS and then cultured under standard conditions at 37°C with 5% CO_2 for 3 days. The cells were imaged using Moticam 480 to observe cellular morphology and growth rate. Similar growth and identical cellular morphology were detected for both the Exosome-depleted FBS using Norgen's FBS Exosome Depletion Kits and the standard FBS

FBS Exosome Depletion Kit I & II (Slurry Format)

Cat No. 61100, 61400

- Deplete exosome-sized vesicles from versatile FBS volumes of up to 280 mL
- No protease treatment required
- No time-consuming ultracentrifugation
- No precipitation reagents required
- No overnight incubation required
- Depleted FBS has no detectable cow's miRNA
- The depleted FBS provides the same cellular growth rates as the standard FBS

Efficient depletion of cow's exosomes from Fetal Bovine Serum

Norgen's FBS Exosome Depletion Kits (Slurry Format) constitute an all-in-one system for the depletion of cow's exosomes from FBS prior to using it as a growth supplement in your culture medium. The FBS recovered from the depletion process is exosome-depleted and does not contain any quantifiable bovine miRNAs. Moreover, the exosome-depleted FBS will support the growth of your cells of interest similar to the non-depleted FBS. Norgen's kits allows for the depletion of different FBS volumes with a maximum volume ranging from 140 mL to 280 mL. The depletion is based on Norgen's proprietary resin. These kits provide a clear advantage over other available kits in that they do not require ultracentrifugation, any special instrumentation, precipitation reagents or any protease treatments. More importantly, the depletion process is an inexpensive method for depletion of your own FBS, as compared to the current ready-to-use exosome-depleted media available on the market.

Versatile sample input up to 280 mL

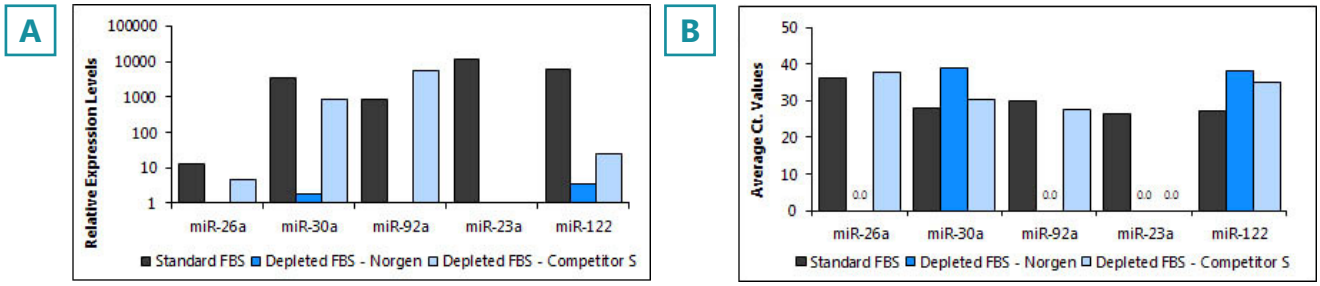
Feature	Specification
Sample Type	Fetal Bovine Serum
Sample Volume Range	Up to 140 mL FBS Exosome Depletion Kit I (Slurry Format) Up to 280 mL FBS Exosome Depletion Kit II (Slurry Format)
Depletion	Deplete exosome-sized vesicle
Bovine miRNA	No detectable bovine miRNA
Time to Complete 6 Purifications	40 minutes

Ordering Information

Description	Size	Cat. Number
FBS Exosome Depletion Kit I (Slurry Format)	6 preps	61100
FBS Exosome Depletion Kit II (Slurry Format)	12 preps	61400

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
SCAN HERE



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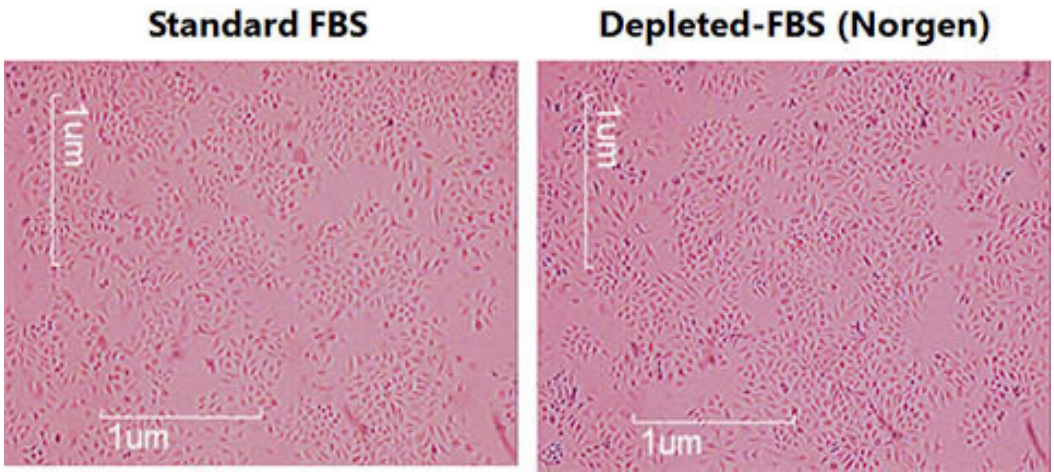

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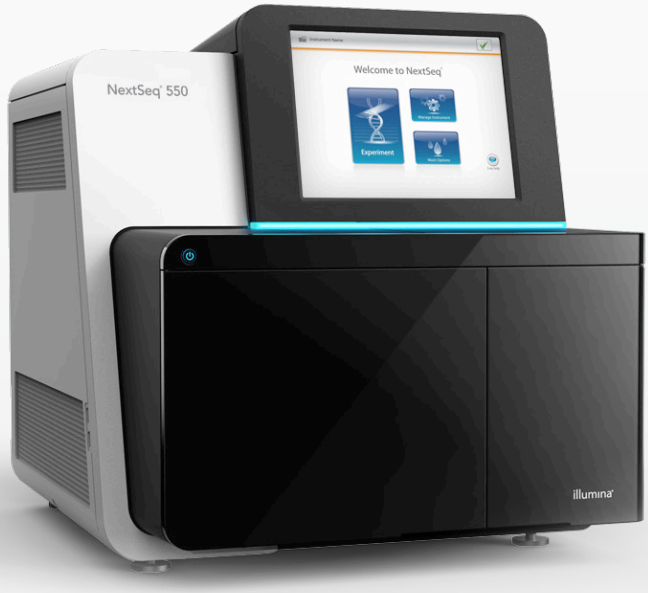


Related Products

Exosomal RNA Isolation

Exosomes Labelling and Cleaning

RNA Clean-up and Concentration

Low abundance RNA quantification

Small RNA Library Prep

Next Generation Sequencing Services

Exosomal RNA Isolation Kit

Cat No. 58000

For use with one of our intact exosome purification kits

- Bind and elute all RNA irrespective of size or GC content, without bias
- No phenol extractions
- No Proteinase K treatment
- No carrier RNA
- Concentrate isolated RNA into a flexible elution volume ranging from 50 µL to 100 µL
- Purify high-quality RNA in 15-20 minutes
- Purified RNA is suitable for a variety of downstream applications, including Small RNA Sequencing. Find out more information on Norgen's NGS services

Isolate all sizes of **exosomal** and **extracellular vesicle RNA, including microRNA**

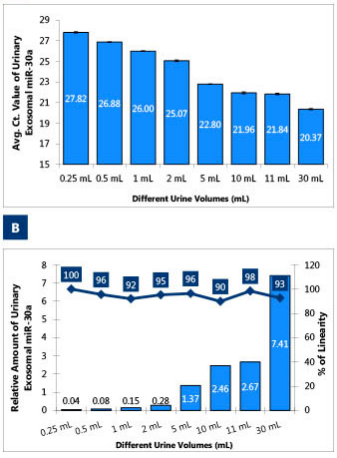


Figure 1. Isolation of RNA from exosomes purified from different urine volumes. Norgen's Exosomal RNA Isolation Kit (Cat# 58000) was used to isolate RNA from exosomes isolated from different urine volumes purified using Norgen's Urine Exosome Purification Kits (Cat# 57700, 57800 and 57900). Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated urinary exosomal miR-30a. (A) The urinary exosomal miR-30a is linearly decreasing with increasing the sample input volume. B) The relative amount of the urinary exosomal miR-30a shows excellent linearity with a percentage of recovery of more than 90%.

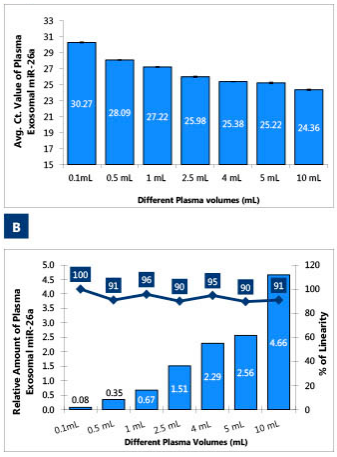


Figure 2. Isolation of RNA from exosomes purified from different plasma volumes. Norgen's Exosomal RNA Isolation Kit (Cat# 58000) was used to isolate RNA from exosomes isolated from different plasma volumes purified using Norgen's Plasma/Serum Exosome Purification Kits (Cat# 57400, 57500 and 57600). Two microlitres of the isolated RNA was then used as the template in RT-qPCR reactions to assess the amplification of the isolated plasma exosomal miR-26a. (A) The plasma exosomal miR-26a is linearly decreasing with increasing the sample input volume. B) The relative amount of the plasma exosomal miR-26a shows excellent linearity with a percentage of recovery of more than 90%.

Ordering Information

Description	Size	Cat. Number
Exosomal RNA Isolation Kit	50 preps	58000

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
SCAN HERE

Exosomes Labelling and Cleaning Kit NEW!

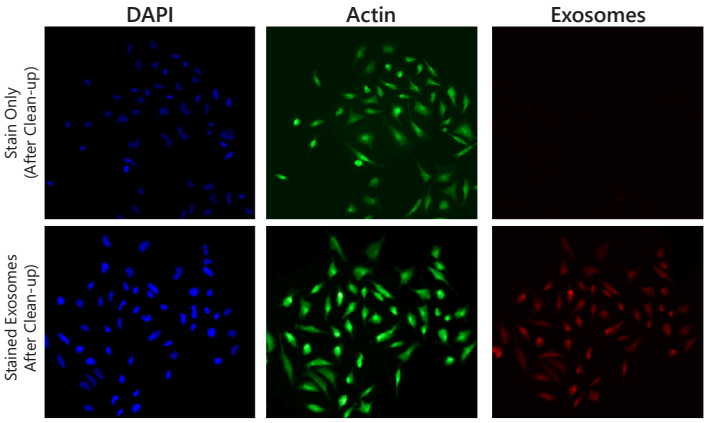
Coming Soon

Norgen's Exosomes Labeling and Cleaning Kit is an all-in-one system allowing for the labeling and clean-up of unbound label of exosomes isolated using various methods and from various biological samples. The cleaning process is based on Norgen's proprietary resin and slurry. Our kit provides a quick and easy way to label and clean exosomes for *in vitro* and *in vivo* analysis.

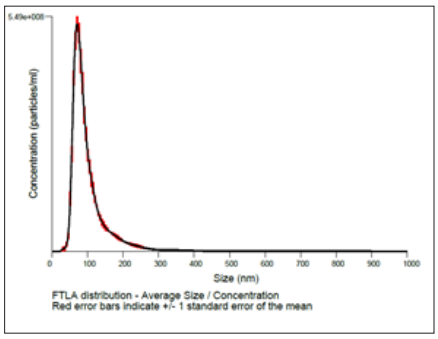
- Allows for efficient labelling of exsosomes with very low levels of background
- Allows for labelling of exosomes isolated using various methods, including Norgen Biotek Corp. proprietary kits, ultracentrifugation and precipitation reagents
- Convenient & fast protocol, which includes both labelling and cleaning procedures
- Excitation at 590 nm/Emission at 617 nm

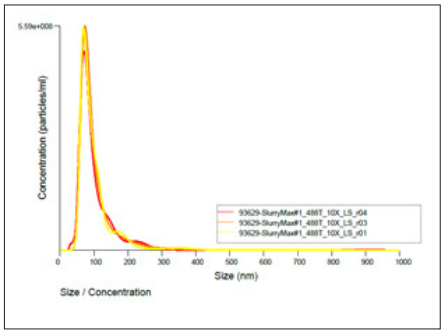
Rapid and efficient **labelling** and **cleaning of exosomes** allowing for visualization with low background

HELA cells and HELA-derived exosomes



Size distribution of the exosomes after labeling





Ordering Information

Description	Size	Cat. Number
Coming Soon!		

RNA Clean-Up and Concentration Micro-Elute Kit

Cat No. 61000

- Concentration of small amounts of RNA into 8 µL
- Ideal for concentrating RNA samples prior to NGS library preparation
- Concentrate from larger elution volumes to more manageable elution volumes
- Ideal for concentrating RNA purified from exosomes, plasma, serum, urine, and other bodily fluids, and any RNA samples initially purified in large volumes
- Efficient RNA cleanup from enzymatic reactions – labeling, DNase treatment and in vitro transcription
- Cleanup of RNA isolated using different methods, including phenol/chloroform extractions
- Fast and easy processing using rapid spin-column format in 15 minutes
- Suitable for all sizes of RNA, including microRNA (miRNA) without bias

For rapid and efficient clean-up and concentration of Total RNA, including microRNA, without phenol from small inputs

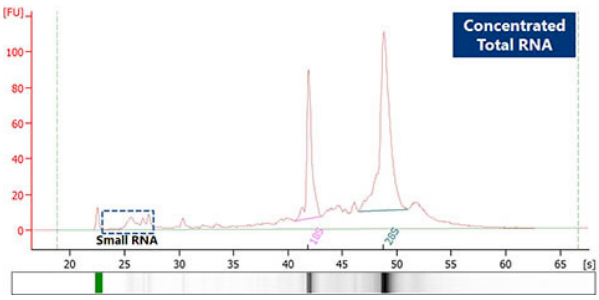


Figure 1. Excellent Quality of Concentrated RNA. Total RNA isolated from HeLa cells (2 µg) was concentrated to 8 µL using the RNA Clean-Up and Concentration Micro-Elute Kit. The excellent quality is indicated by the electropherogram generated using the Agilent 2100 Bioanalyzer (RIN > 9). The concentrated RNA is a true 'total RNA' as can be observed by the presence of small RNA species.

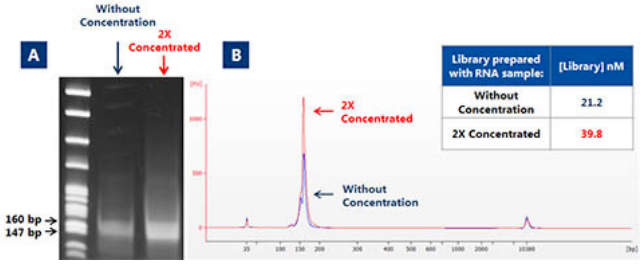


Figure 2. Concentration of RNA prior to Next Generation Sequencing (NGS) applications. Total RNA was purified from 200 µL of plasma collected on EDTA blood tubes using Norgen's Total RNA Purification Kit (Cat # 17200) and eluted in 50 µL of elution solution. The same RNA was also concentrated two-fold using the Micro-Elute RNA Column by eluting in 25 µL of elution solution. Five microliters of both the RNA without additional concentration and the 2X concentrated RNA were used as inputs to generate RNA libraries (using the NEBNext® Small RNA Library Prep Set for Illumina® and following manufacturer's instructions) for small RNA NGS on the MiSeq (Illumina) platform. A) The prepared small RNA libraries were visualized on a 6% TBE polyacrylamide gel, where the library prepared with 2X concentrated RNA contained more ligated/indexed miRNA cDNA (147-160 bp) products than the library prepared using the RNA without concentration. B) The cDNA was extracted from excised gel bands and interrogated using the Agilent 2100 Bioanalyzer (High Sensitivity DNA Assay). As would be expected based on input, the small RNA library prepared with the 2X concentrated RNA sample was approximately two times more concentrated than the library prepared with RNA without prior concentration (39.8 vs 21.2 nM, respectively).

Ordering Information

Description	Size	Cat. No
RNA Clean-Up and Concentration Micro-Elute Kit	50 preps	61000

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
SCAN HERE

Low Abundance RNA Quantification Kit

Cat No. 58900

- Quantify RNA of a wide spectrum of concentrations, including the lower ng per µL, pg per µL and sub-pg per µL range
- Compatible with any Real-Time PCR system
- RNA is accurately quantified using a standard curve constructed from the provided RNA standard

Quantify Low Abundant RNA from Exosomes & more with ease

Sensitivity of Norgen's Kit Compared to Other Methods

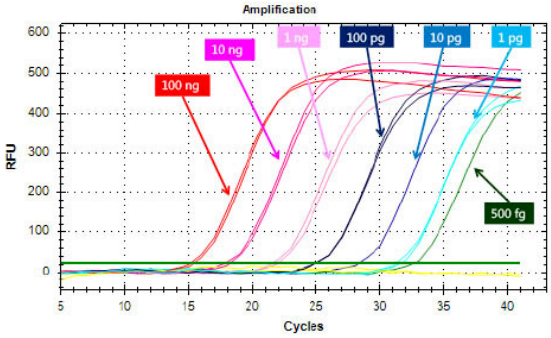


Figure 1. Sensitivity of RNA Quantification in the Picogram Range using the Low Abundance RNA Quantification Kit. A representative qPCR Baseline Graph showing the amplification of an RNA standard dilution series. The Low Abundance RNA Quantification Kit can quantify purified RNA from low abundance samples such as liquid biopsies (plasma or urine). As little as 500 fg of RNA can be quantified using Norgen's kit.

Sensitive RNA Quantification from Small Volumes of Plasma

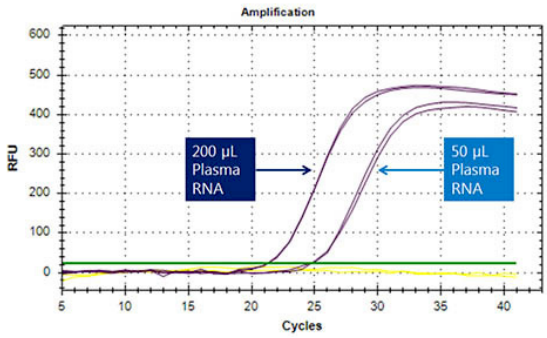


Figure 2. Sensitivity of RNA Quantification from Small Volumes of Human Plasma using the Low Abundance RNA Quantification Kit. A representative qPCR Baseline Graph showing the amplification of total plasma RNA isolated from either 50 or 200 µL of human plasma using Norgen's Plasma/Serum RNA Purification Mini Kit (Cat# 55000). The Low Abundance RNA Quantification Kit could quantify purified RNA from such low abundance samples (and others such as urine, exosomes etc) with purified RNA concentrations that are 100 pg per µL or less.

Ordering Information

Description	Size	Cat. Number
Low Abundance RNA Quantification Kit	48 rxns	58900

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
SCAN HERE

Small RNA Library Prep Kit for Illumina

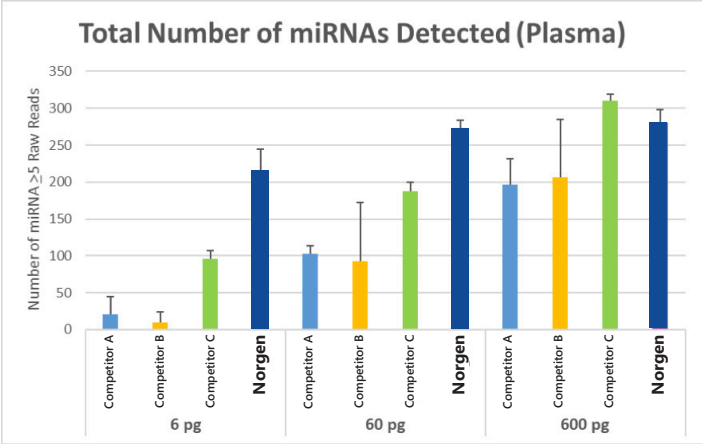
Cat No. 63600, 63610, 63620

- Complete kits with reagents for all steps from 3′ ligation through size selection
- Includes all clean-up columns and reagents in one convenient kit – **no need to purchase several kits**
- Tremendous time savings with size selection without gels and lengthy elution steps
- Enhancement of useful small RNA detection (3-4x increase) in human plasma/serum by depleting an abundant sequence that may occupy up to 50% of NGS reads

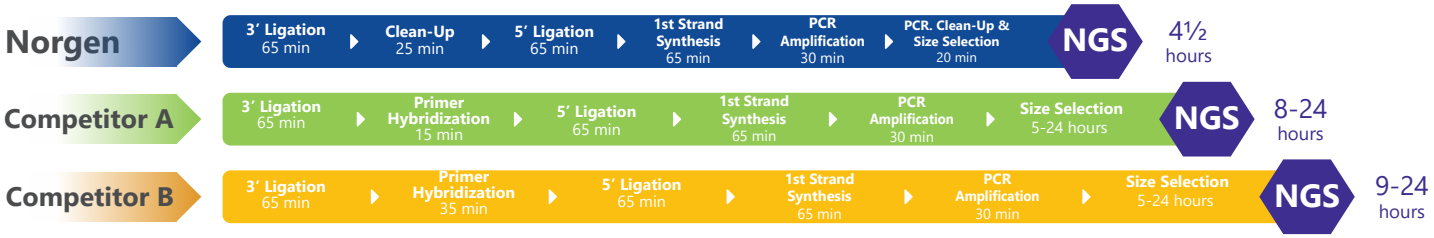
Optimized for **ultra-low** RNA inputs

Our workflow, using Norgen's Small Library Prep Kit for Illumina, will yield higher numbers of microRNA detected when compared to competitor's workflows. Norgen's Small RNA Library Kit exhibits outstanding performance, specifically when working with ultra-low input samples.

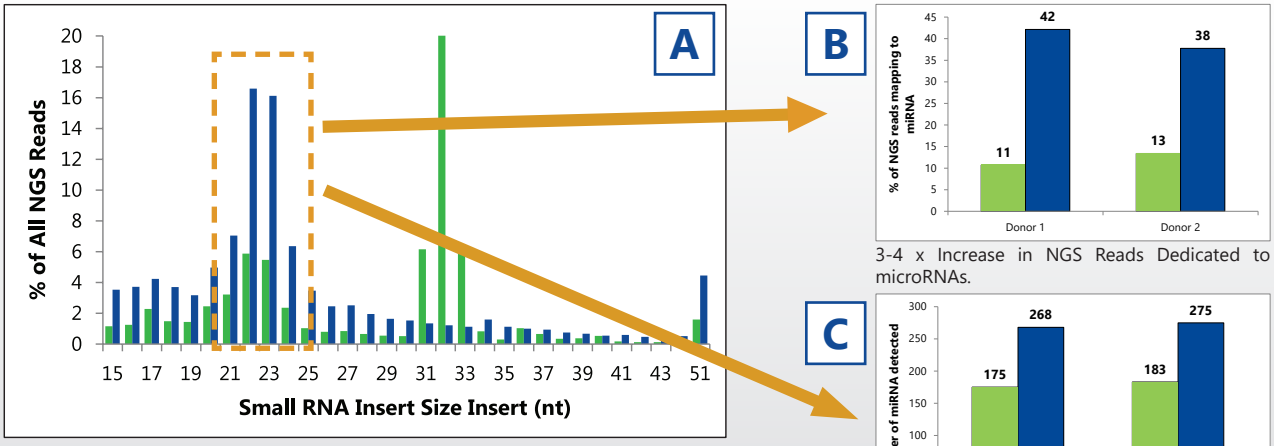
- Dilution series of purified plasma RNA used for different vendor's library prep kits
- Sequenced at equal molarity at ~ 1.5-2M Total Raw Reads per sample
- Norgen workflow showed much higher number of miRNA detected at ≥ 5 raw reads across all concentration tested



Workflow Comparisons



Related Kit Data



3-4 x Increase in NGS Reads Dedicated to microRNAs.

50% Increase in microRNA Detected.

Figure 1. Norgen's Abundant Sequence Depletion Kit (For RNA Purified from Human Plasma/Serum) specifically removes an abundant RNA in human plasma/serum that usually takes up > 50% reads of a standard Small RNA-Seq run (see green bars, Panel A) resulting in microRNA-related reads in < 10% of the reads. With the depletion (blue bars, Panel A), the reads dedicated to microRNA significantly increase to > 40% (Panel B), resulting in more microRNA detected (Panel C).

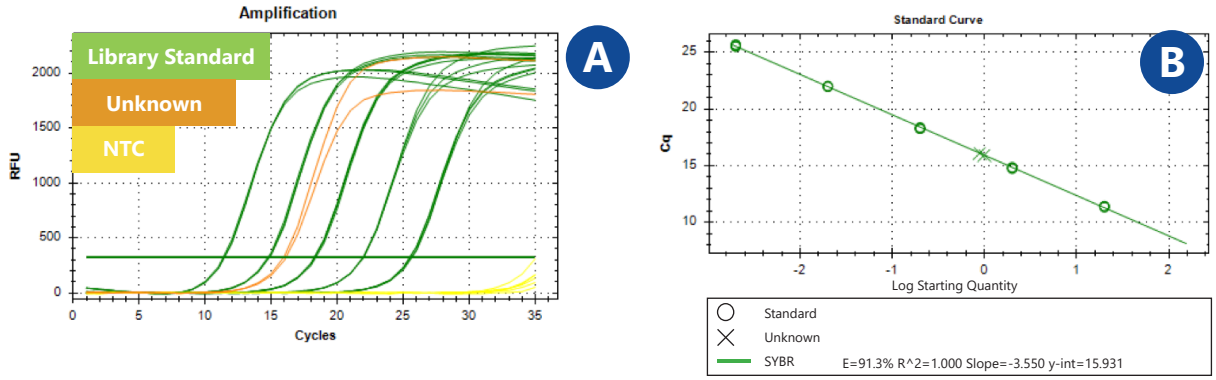


Figure 2. A representative qPCR baseline graph showing the successful amplification of Quantified NGS Library Standards (Green) with a range from 20 pM to 2 fM, using Norgen's NGS Library Quantification Kit (for Small RNA-Seq) (Panel A.) Duplicate amplification of a sample Small RNA-Seq library (at 1:10,000 dilution) was performed (Orange). The derived library concentration was 9.41 nM. Norgen's NGS Library Quantification Kit (for Small RNA-Seq) is of good quality as shown with the high PCR efficiency and correlation in the standard curve (Panel B) with low background singals (No Template Control - NTC as Yellow in Panel A)

Ordering Information

Description	Size	Cat. No
Small RNA Library Prep Kit for Illumina	6 rxns	63610
Small RNA Library Prep Kit for Illumina (Indexes 1-24)	24 rxns	63600
Small RNA Library Prep Kit for Illumina (Indexes 25-48)	24 rxns	63620

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

For more information
SCAN HERE

Next Generation Sequencing Services

for Small RNA and microRNA

Norgen Biotek offers comprehensive services for Next-Generation Sequencing (NGS) in an accredited state-of-the-art laboratory from sample isolation to sequencing and bioinformatics analysis. We have extensive expertise in sample preparation, sequencing and analysis of all types of samples, specializing in ultra-low input samples including liquid biopsies (plasma/serum, urine and exosomes).

for Liquid Biopsies (Plasma/Serum, Urine and Exosomes), FFPE, Cells and Tissues

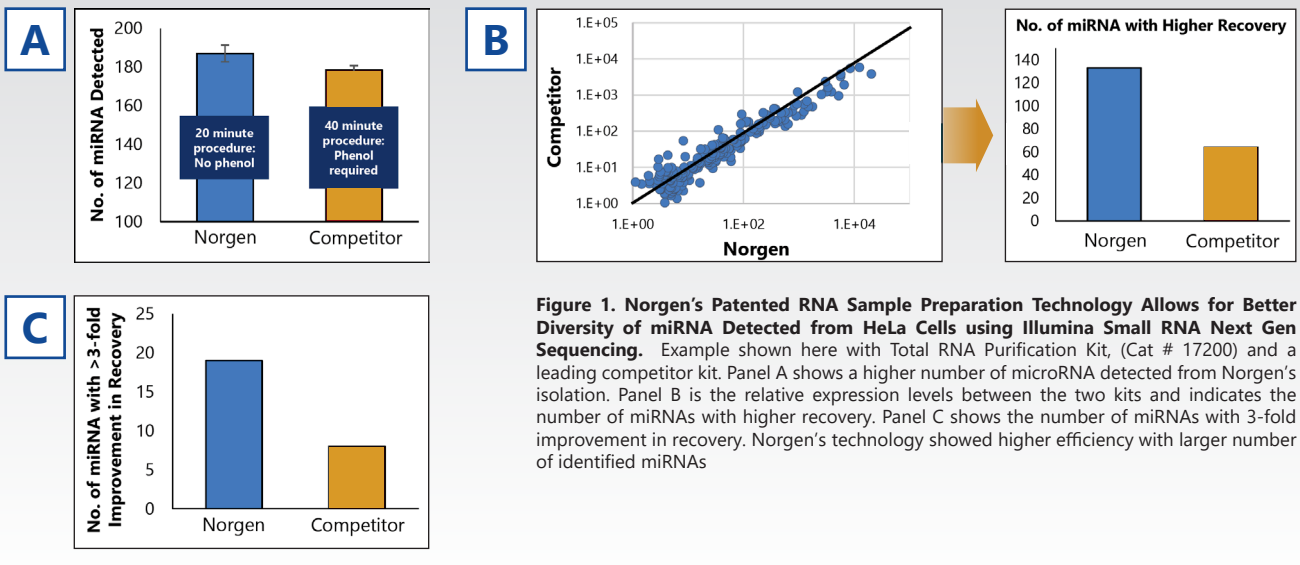


Figure 1. Norgen's Patented RNA Sample Preparation Technology Allows for Better Diversity of miRNA Detected from HeLa Cells using Illumina Small RNA Next Gen Sequencing. Example shown here with Total RNA Purification Kit, (Cat # 17200) and a leading competitor kit. Panel A shows a higher number of microRNA detected from Norgen's isolation. Panel B is the relative expression levels between the two kits and indicates the number of miRNAs with higher recovery. Panel C shows the number of miRNAs with 3-fold improvement in recovery. Norgen's technology showed higher efficiency with larger number of identified miRNAs

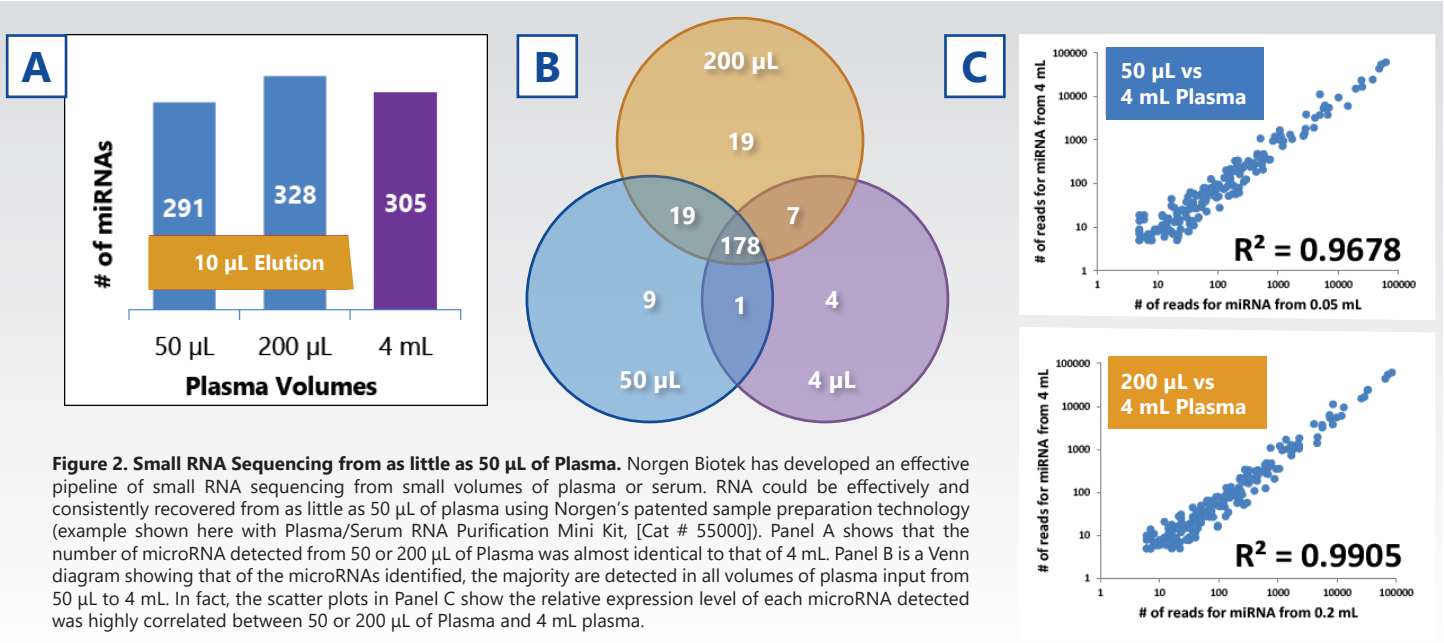


Figure 2. Small RNA Sequencing from as little as 50 µL of Plasma. Norgen Biotek has developed an effective pipeline of small RNA sequencing from small volumes of plasma or serum. RNA could be effectively and consistently recovered from as little as 50 µL of plasma using Norgen's patented sample preparation technology (example shown here with Plasma/Serum RNA Purification Mini Kit, [Cat # 55000]). Panel A shows that the number of microRNA detected from 50 or 200 µL of Plasma was almost identical to that of 4 mL. Panel B is a Venn diagram showing that of the microRNAs identified, the majority are detected in all volumes of plasma input from 50 µL to 4 mL. In fact, the scatter plots in Panel C show the relative expression level of each microRNA detected was highly correlated between 50 or 200 µL of Plasma and 4 mL plasma.

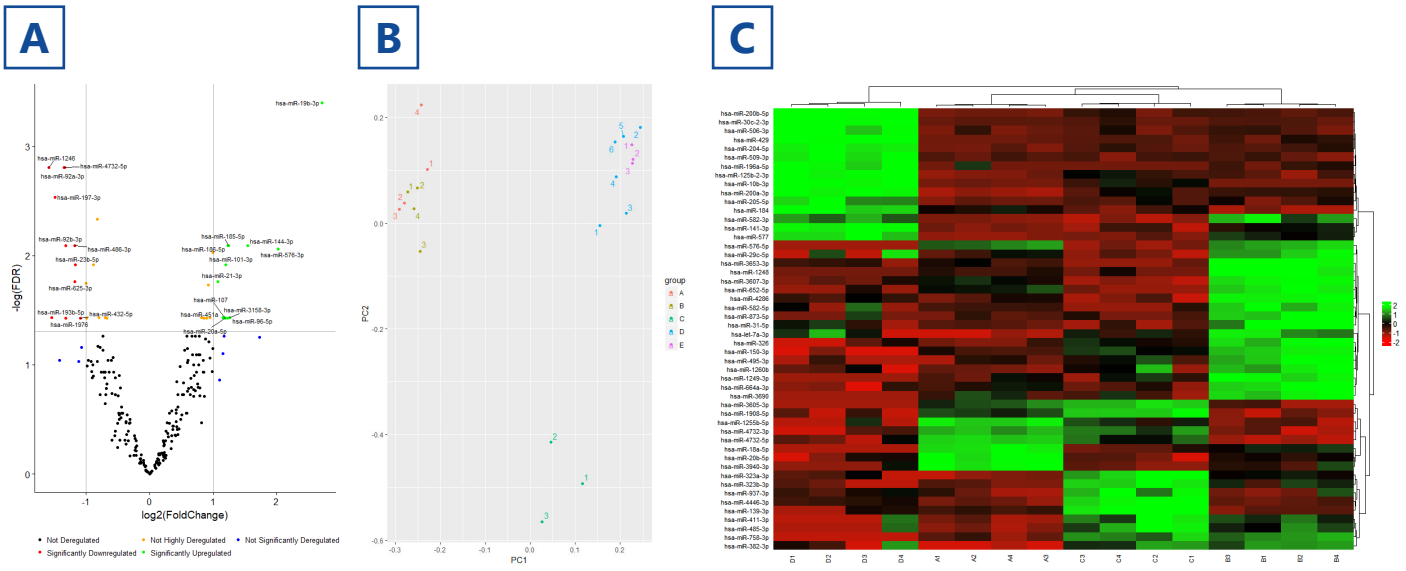


Figure 3. Norgen's Data Analysis provides statistically significant differential expression information between conditions and displays the data in various illustrations. Panel A shows the volcano plot that illustrates the relation between –log10 FDR and log2 fold change between the control and indicates upregulated and downregulated miRNAs. Panel B is the principal component analysis (PCA) plot that illustrates the distance between samples based on miRNA profile. Samples are clustered in the PCA plot based on their biology unless other factors, such as sample purity, are causing a more pronounced source of variation. Panel C is a Heat map with hierarchal clustering of miRNAs and samples. The color scale indicates the relative expression level of a miRNA to the mean, where green and red indicate higher or lower expression, respectively.

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NORGEN BIOTEK CORP.

LUBIO SCIENCE

Your distributor in Switzerland

LubioScience GmbH
Baumackerstrasse 24
8050 Zürich

+41 (0)41 417 02 80

info@lubio.ch
www.lubio.ch

Ordering Information

NORGEN BIOTEK CORP.

Norgen Biotek Corp.
3430 Schmon Pkwy
Thorold, ON
Canada L2V 4Y6

Website:	www.norgenbiotek.com
Email:	orders@norgenbiotek.com
Toll-Free (North America):	1-866-NORGENB
Telephone:	905-227-8848
Fax:	905-227-1061

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