The five advantages of silicon-carbide based RNA extraction

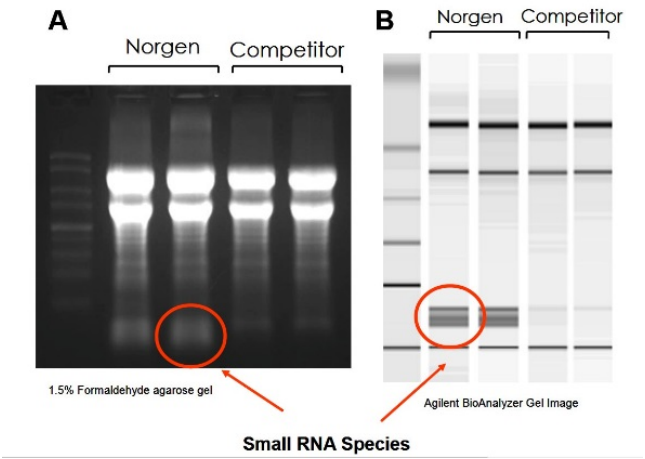
Study of gene expression using RNA has played an important role in better understanding the biology of organisms, from developmental timing and stem cell differentiation to oncogenesis and diseases. However, with the discovery of smaller RNAs and the emergence of the field of RNA interference, it has become indispensable to successfully isolate total RNA without any kind of bias. RNA isolation by earlier and traditional methods, such as the TRIZOL method, rely on harmful organic substances. While this method successfully captures large and small RNAs, it is time-consuming and working with harmful substances is best avoided if possible. As a result, spin columns containing silica-fiber or silica-matrix came onto the market and were a safer and faster alternative. They are still available from many suppliers today.

Unfortunately, silica-based methods have a big downside: a bias in the kind of RNA extracted. Silica columns preferentially extract RNA with a high GC content and are also very bad at isolating small RNAs below 200nt. This can be disastrous for studies on RNA interference and posttranslational regulation. Thus, in order to capture smaller RNAs successfully, additional steps have to be included, removing one of the main advantages of the silica-based method, namely the ease of use. As a result, there is a **demand for a method that combines the ease of use of spin column chromatography with the RNA species diversity** of TRIZOL methods.

Norgen's silicon-carbide (SiC) technology fulfills this demand and offers the following advantages:

Broad spectrum RNA binding

As mentioned above, silica-based technology exhibits a bias towards capturing RNA with high GC content, and RNA fragments with a high molecular weight. In contrast, silicon-carbide technology has demonstrated a uniform binding affinity for all RNA species (including small RNAs; 200 nt or smaller). This offers researchers and clinicians a more complete picture of the sample's true RNA profile thus avoiding any false-negative results that could be caused due to the bias exhibited by the silica-based technology.



Norgen's silicon-carbide-based kit successfully isolates total RNA including small RNAs of under 200nt, while the competition's silica-based kits only capture larger RNAs.

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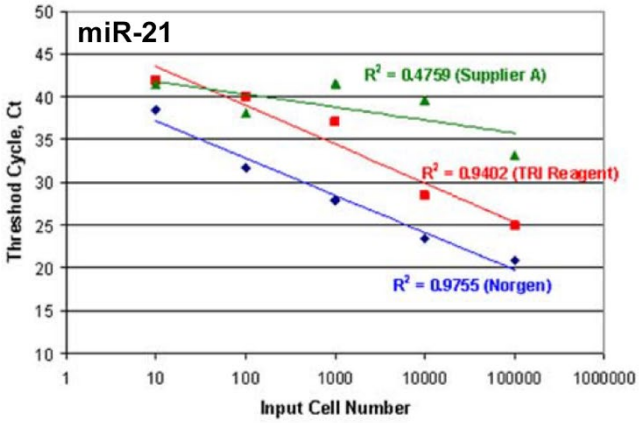
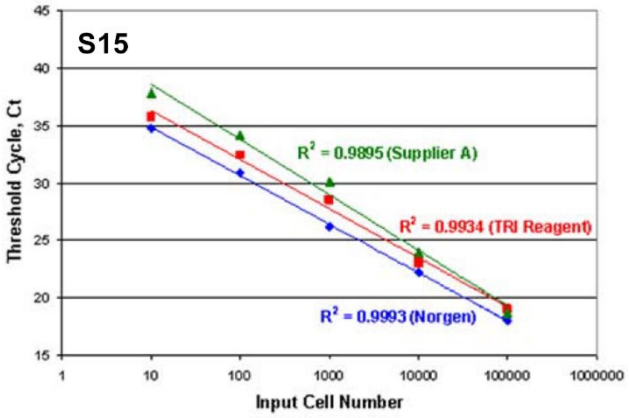
High sensitivity

In comparison to total RNA isolation by silica, using SiC shows better linearity and higher sensitivity for both large mRNAs and miRNAs. This is currently especially important, as the sensitivity of most commercially available diagnostic viral qRT-PCR detection kits are highly affected by the RNA quality that is used for viral detection. Norgen's SiC technology yields viral RNA with the highest quality enabling the detection of as low as 400 copies/mL of saliva (i.e. 10 viral copies/PCR reaction). This is ideal for the detection of ultra-low viral loads particularly in asymptomatic and recovering patient samples.





The fact that SiC technology doesn't exhibit any bias towards RNA sizes/sequences is especially advantageous as nucleic acids stored in viral transport media (VTM) can degrade over time. This generates fragmented viral RNA that will be efficiently captured using Norgen's SiC technology. In contrast, silica-based technology won't be able to efficiently capture such fragmented viral RNA causing false-negative results. The use of SiC technology ensures the accurate detection of SARS-CoV-2 regardless of time-dependent viral RNA degradation.

Total RNA isolated using SiC showed better linearity and higher sensitivity for both large mRNAs and miRNAs.

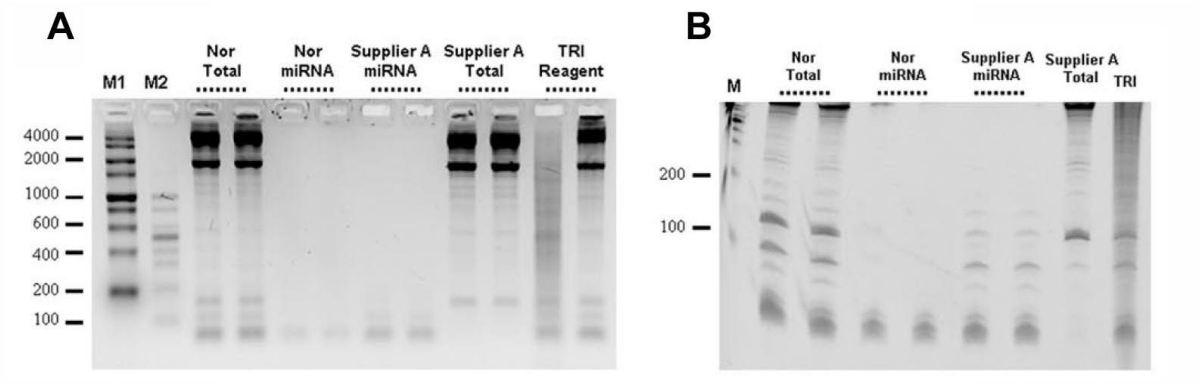
Total RNA was isolated from increasing amounts of HeLa cells using Norgen's SiC-based total RNA purification kit (*blue*), a competitor's silica-fiber-based total RNA purification kit (*green*), and guanidine thiocyanate/ phenol-based TRI reagent as control. Then RT-qPCR was performed to detect *miR-21* (*top*) and *S15* (*bottom*).

Carrier RNA-free extraction

To enhance the RNA binding efficiency of silica-based technology for capturing ultra-low RNA input, poly(A) carrier RNA is commonly used in commercially available viral RNA extraction kits. The presence of carrier RNA in the eluate can severely impact sensitive downstream applications like RNAseq by significantly reducing the sequencing efficiency of target sequences. Due to the high sensitivity of Norgen's SiC technology of capturing ultra-low RNA inputs, an RNA carrier is not required, thus enhancing the sequencing efficiency of target sequences.

Phenol/chloroform-free extraction

The use of hazardous chemicals for extracting nucleic acids, such as phenol/chloroform, can be laborious and are not amenable to high-throughput processing. Moreover, the quality of the RNA extracted using phenol/chloroform may not be high enough for sensitive down-stream applications. Norgen's SiC technology does not utilize phenol/chloroform or any hazardous organic chemicals, and hence the quality of the extracted RNA will be optimal for any sensitive down-stream application.



Total RNA was isolated from one million HeLa cells using Norgen's SiC (*Nor Total*) or silica-fiber (*Supplier A Total*). In addition, RNA was isolated using two commercially available miRNA kits from the same suppliers, and a TRI reagent method for comparison. The isolated RNA was then resolved on a 1.5% formaldehyde-agarose gel *(A)* and on an 8% Urea-PAGE gel *(B)*. Only total RNA using SiC or TRI contained small RNA species. The enrichment of miRNA with Norgen's SiC kit did not need any phenol extractions, unlike the miRNA enrichment using silica fiber.

Virus inactivating buffers

The use of universal transport media (UTM) and viral transport media (VTM) is a common practice in disease screening programs. However, samples stored in these media leave medical laboratory technicians potentially exposed to infectious samples. RNA extractions that include lytic buffers like Norgen's Lysis Buffer A and Buffer RL are ideal for sampling methods that utilize UTMs and VTMs as they render these samples non-infectious.

In conclusion, Norgen's RNA extraction kits offer the following five advantages:

1) **Broad spectrum RNA binding**
2) **High sensitivity**
3) **Carrier-RNA-free extraction**
4) **Phenol-chloroform-free extraction**
5) **Virus inactivating buffers**

For more information on products, please visit us at www.lubio.ch/rna-purification or contact us at info@lubio.ch.