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How to perform DNA gel electrophoresis

Agarose gel preparation - Gel staining - Gel electrophoresis

Introduction

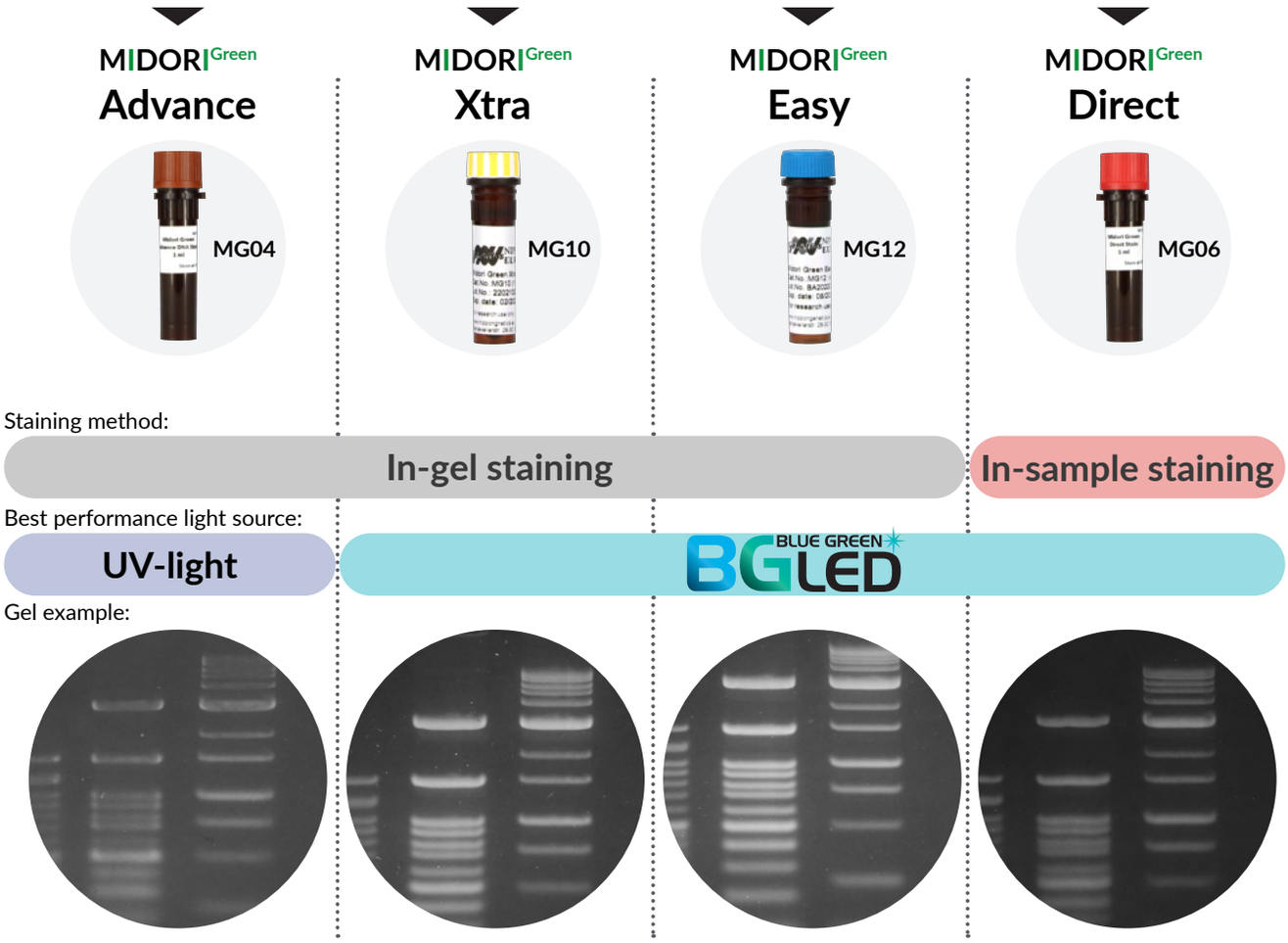
DNA electrophoresis is a standard method for separating and analyzing DNA fragments according to their size. The DNA sample is applied to an agarose gel together with a DNA dye. After voltage application in the electrophoresis device, the DNA migrates towards the positive pole due to its negative charge. During migration through the agarose, the DNA is separated based on its fragment size, as smaller fragments migrate through the gel matrix faster than larger fragments. After the separation, the DNA bands can be visualized in the agarose gel with a suitable detection light source. The gel with the visualized DNA bands can now be analyzed with a gel documentation system, or DNA bands can be cut out of the gel for further preparation steps.

DNA gel electrophoresis product information

Cat. No.	Product	Content
Electrophoresis Chamber		
MU2	Mupid™-One	Mupid™-One electrophoresis system with 1x gel chamber, 1x power controller, 1x gel casting set, 4x combs, 2x gel trays S, 1x gel tray L
MU4	Mupid™-One LED Illuminator	Mupid™-One LED Illuminator
Running Buffer		
ID1521	50x TAE Buffer	500 ml
ID1531	10x TBE Buffer	500 ml
Agarose		
AG01	FastGene® Agarose	100 g powder
AG02	FastGene® Agarose	500 g powder
AG05-100	FastGene® Agarose Tablets	100 Tablets (0.5 g Agarose per tablet)
AG09	MIDORI^{Green} Advance TBE Agarose Tablets	100 Tablets
AG10	MIDORI^{Green} Advance TAE Agarose Tablets	100 Tablets
AG11	MIDORI^{Green} Advance Agarose Tablets (without buffer)	100 Tablets
AG12	MIDORI^{Green} Xtra Agarose Tablets (without buffer)	100 Tablets
AG13	MIDORI^{Green} Xtra TAE Agarose Tablets	100 Tablets
FG-830	FastGene® Agarose Gel Band Cutter	50 Units
Safe DNA Stain		
MG04	MIDORI^{Green} Advance	1 ml (25.000x - for staining 25 l of agarose)
MG10	MIDORI^{Green} Xtra	1 ml (25.000x - for staining 25 l of agarose)
MG12	MIDORI^{Green} Easy	0.4 ml (10.000x - for staining 4 l of agarose)
MG06	MIDORI^{Green} Direct (with loading dye)	1 ml (10x conc. for direct use in sample)
DNA Marker / Loading Buffer		
MWD50	FastGene® 50 bp Standard DNA Marker	500 µl
MWD100	FastGene® 100 bp Standard DNA Marker	500 µl
MWD1P	FastGene® 1 kb Standard DNA Marker Plus	500 µl
ID1654	6x Nucleic Acid Loading Buffer	10 ml

Safe DNA stain - The MIDORI[Green] Family

Our set of MIDORI[Green] dyes offers a perfect solution for different staining preparation or gel documentation conditions. MIDORI[Green] Advance, MIDORI[Green] Xtra and MIDORI[Green] Easy are added to the melted agarose for gel staining. If direct addition of the dye to the samples is preferred, MIDORI[Green] Direct is the stain of choice. While MIDORI[Green] Advance shows the strongest DNA signals with UV-light, MIDORI[Green] Xtra, MIDORI[Green] Easy and MIDORI[Green] Direct perform best with visible light and especially our Blue/Green LED light technology. No matter which MIDORI[Green] dye you use for your applications, all give excellent DNA signals and are completely safe to use, as certified by our external safety reports.



Protocol: How to perform DNA gel electrophoresis

Description

This protocol describes how to perform a typical standard DNA gel electrophoresis workflow with NIPPON Genetics EUROPE products. It shows the complete procedure starting from TAE running buffer preparation, gel preparation from agarose powder, MIDORI^{Green} DNA staining and gel electrophoresis with the MUPID^{TM}-ONE system. This protocol is meant to be used as a quick guide. Please refer to the product manuals for additional and more detailed information.

Required items

Product	NGE Catalog number
FastGene® Agarose	AG01 / AG02
50x TAE Buffer	ID1521
Erlenmeyer flask (100 - 250 ml)*	---
Scale*	---
Microwave*	---
MIDORI^{Green} DNA Stain	MG04 / MG10 / MG12 / MG06
Pipette (10 µl / 20 µl)*	---
Pipette tips (10 µl / 20 µl)**	FG-FT10 / FG-FT20 FG-TP10 / FG-TP20
6x DNA loading Buffer**	ID1654
FastGene® DNA Marker**	MWD50 / MWD100 / MWDP1
Mupid^{TM}-One Gel casting accessories	MU2
Mupid^{TM}-One Electrophoresis System	
Mupid^{TM}-One LED Illuminator	MU4 (optional)
Transilluminator / Gel Documentation System**	Blue/Green LED light system UV light system

*Available from other manufacturers
**Recommended from NGE, but can also be used from other manufacturers

1. Agarose Gel preparation

1.1 Preparation of electrophoresis buffer (TAE)

- Prepare 1 L of 1X TAE electrophoresis buffer by diluting the 50 X TAE stock solution (ID1521) with water (preferably ddH_2O)
- For 1 L of 1X TAE, add 980 ml of (dd)H_2O to 20 ml of 50 X TAE stock solution.

1.2 Agarose powder weighing

- Take the FastGene® Agarose powder (AG01 / AG02) and weigh the appropriate amount of agarose powder on a scale.
- Refer to the table below for the required amount of agarose powder. 50 ml gel are needed for one small gel and 100 ml gel are needed for one large gel.

Gel percentage	Agarose amount	
	50 ml gel	100 ml gel
1.0 %	0.5 g	1.0 g
1.2 %	0.6 g	1.2 g
1.3 %	0.65 g	1.3 g
1.5 %	0.75 g	1.5 g
1.8 %	0.9 g	1.8 g
2.0 %	1.0 g	2.0 g

- Transfer the powder into an Erlenmeyer flask that is 2 to 4 times the volume of the solution being prepared and add the required volume of previously prepared 1X TAE electrophoresis buffer.
- Heat the slurry in a microwave on high power until it starts to boil, allow boiling for 30 sec.
- Remove the flask from microwave, swirl gently to dissolve any remaining agarose particles.
- Reheat on high power for 1-2 minutes, or until the solution is clear and all particles are dissolved.
- Remove the flask from the microwav, and gently swirl it.

Caution when handling the solution: the agarose solution and flask are extremely hot.

2. Gel staining (MIDORI^{Green} Advance/Xtra/Easy)

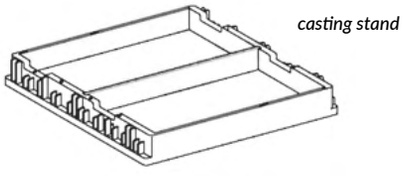
Skip this step if MIDORI^{Green} Direct is used

- After heating and completely dissolving the agarose, take out the flask from the microwave.
- The MIDORI^{Green} **Advance/Xtra** staining solution is **25,000 x** concentrated.
- The MIDORI^{Green} **Easy** staining solution is **10,000 x** concentrated.
- Take a 10-20 µl pipette with a pipette tip and add the required amount of MIDORI^{Green} stain to the dissolved agarose solution. (E.g. add 2 µl MIDORI^{Green} Advance/Xtra to 50 ml agarose solution, or 5 µl MIDORI^{Green} Easy to 50 ml agarose solution.)
- Swirl the flask gently to evenly disperse the MIDORI^{Green} stain in the agarose solution.

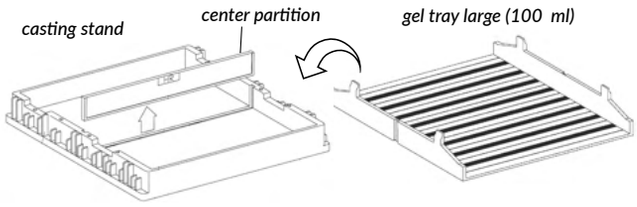
3. Gel electrophoresis (MUPID™-ONE)

3.1 Gel casting assembly

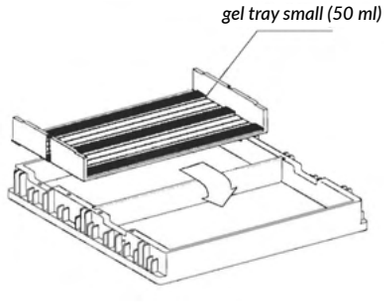
- First, assemble the gel casting accessories from the MUPID™-ONE package.
- Use the casting stand to place the gel trays in it.



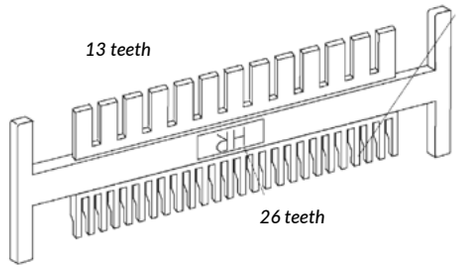
- For casting large 100 ml gels remove the center partition from the casting stand and insert the large gel tray.



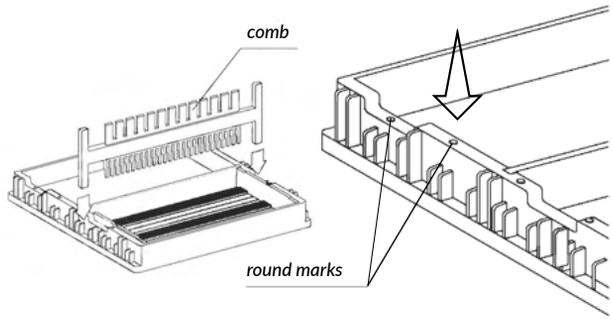
- For casting 50 ml gels, insert the center partition into the casting stand and insert 1 to 2 small gel trays.



- Select either the 13 teeth (6 mm wide wells) or 26 teeth (2 mm wide wells) comb, according to the experimental requirements. The spacing of these wells is also compatible with a multichannel pipette.

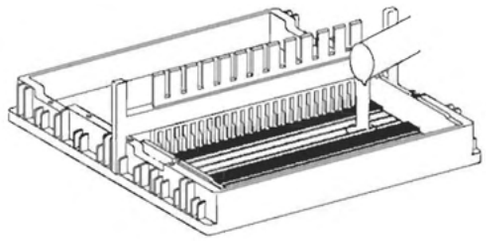


- Place the comb into the casting stand. Place it in positions with the round marks of the stand to obtain equal intervals.



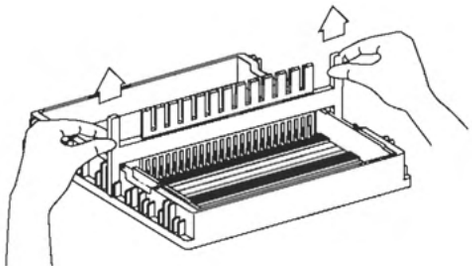
3.2 Agarose gel pouring

- Pour the (stained) agarose solution into the gel tray. 50 ml of agarose solution will lead to a gel thickness of 4 mm.



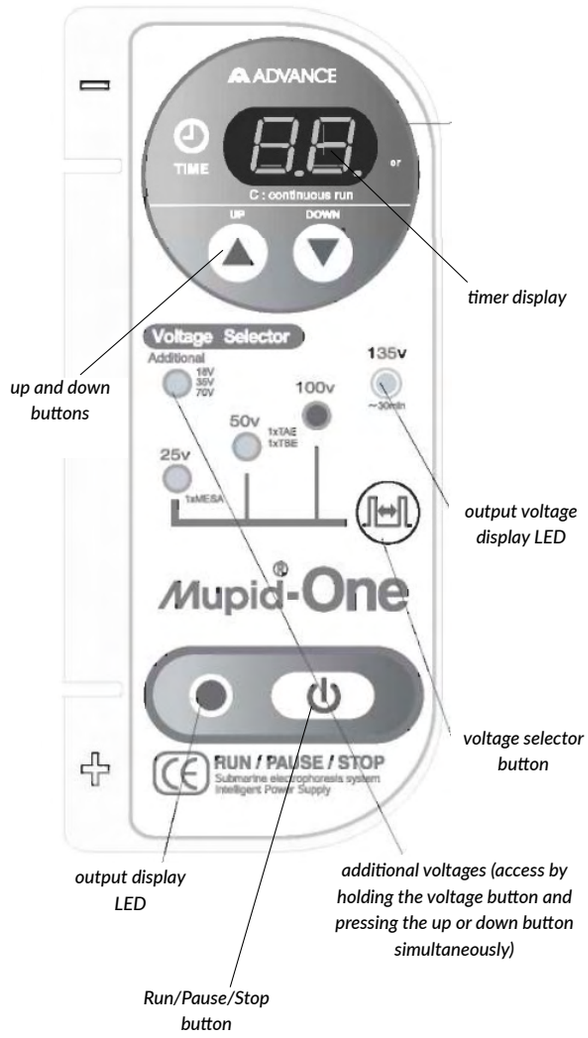
Make sure there are no air bubbles after pouring, especially around the comb. Remove them with a pipette tip, while the agarose gel is still liquid.
It may happen that some gel solution flows under the gel tray. This is not a problem as only very small amounts of gel are involved. It has no influence on the outcome of the experiment.

- Allow the gel solution to solidify for ~20 min at room temperature. It may take longer with lower agarose concentrations.
- Remove the comb(s) with both hands by gently and carefully lifting them upwards. Make sure that the gel is completely solidified around the wells and the wells are free of air bubbles.



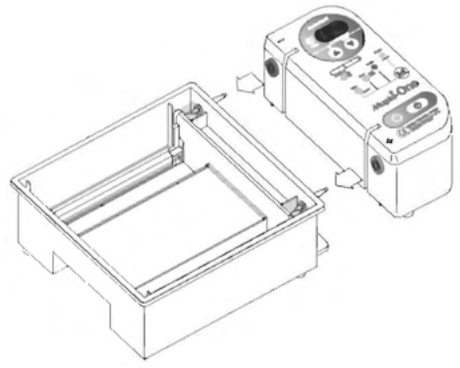
- Hold the gel tray at the notches on both sides of the casting stand and pull the gel tray upwards, out of the casting stand.
- The gel can now be used for DNA electrophoresis.

3.3 MUPID™-ONE functions overview

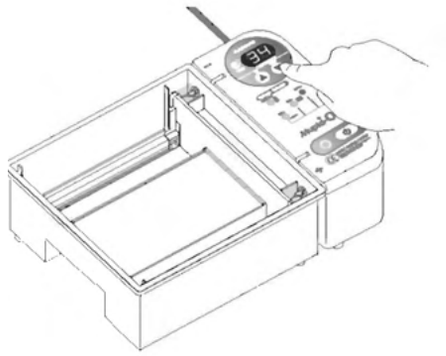


3.4 MUPID™-ONE electrophoresis cell assembly

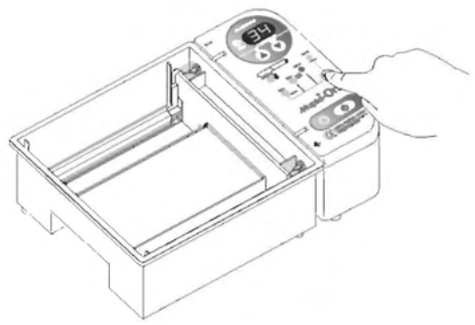
- Place the MUPID™-ONE electrophoresis system on a level surface or table.
- Connect the power supply to the electrophoresis cell.



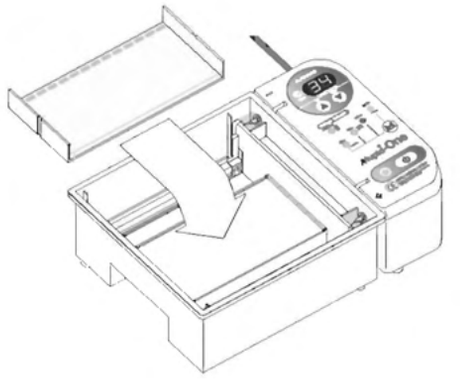
- Connect the power supply via the power cable into a wall outlet. Switch it on, via the red power button.
- Set the timer with the up and down buttons. It can be set between 1 - 99 min. Set 0 for continuous mode („c“ will appear on the display panel.)
- A good starting point is to set the running time to ~30 min.



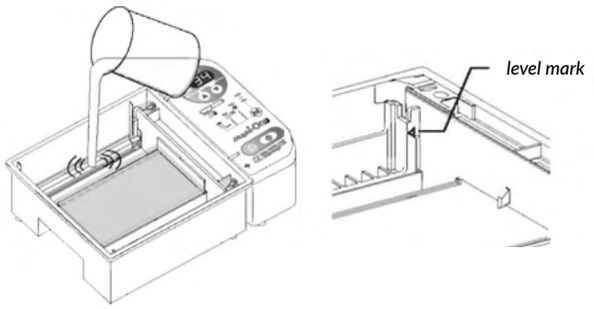
- Select the required output voltage by pressing the voltage selector button. You can choose between 25 V, 50 V, 100 V and 135 V. Additional voltages (18 V, 35 V, 70V) can be accessed by holding the voltage button and pressing the up or down button simultaneously.
- A good starting point is to set the output voltage to 100 V.



- Place the previously prepared agarose gel onto the gel platform in the middle of the electrophoresis cell. Do not remove the gel from the gel tray.
- If using a large gel (100 ml) place the tray in the middle so that its center groove fits the rib of the tank.
- Make sure the gel does not slide out of the gel tray.



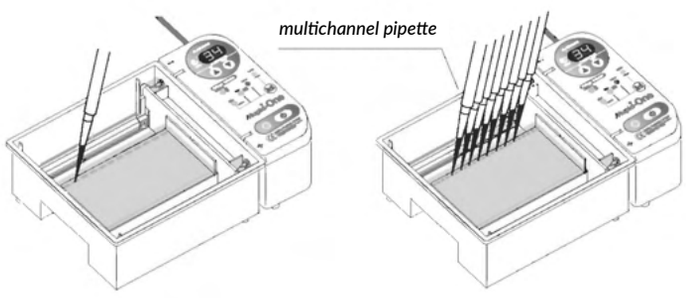
- Carefully pour the previously prepared 1X TAE electrophoresis buffer into the electrophoresis tank.
- Make sure to pour the right amount of running buffer, indicated by the level marks on the inside of the electrophoresis tank. The marks correspond to 340 - 360 ml when using a small gel tray, and to 310 - 330 ml when using a large gel tray.



3.5(1) Sample application

Skip this step if MIDORI^{Green} Direct is used

- Prepare the DNA samples by adding a loading buffer (e.g. 6x DNA loading buffer: ID1657) to each DNA sample.
- Apply the desired amount of sample into the sample wells with a pipette.
- Approximately 12 µl of sample can be loaded into each 6 mm wide well, and approximately 4 µl can be loaded into each 2 mm wide well.
- Also apply ~5 µl of a DNA Marker (e.g. FastGene® DNA Marker: MWD50 / MWD100 / MWDP1).
- Samples can also be applied with a multichannel pipette.

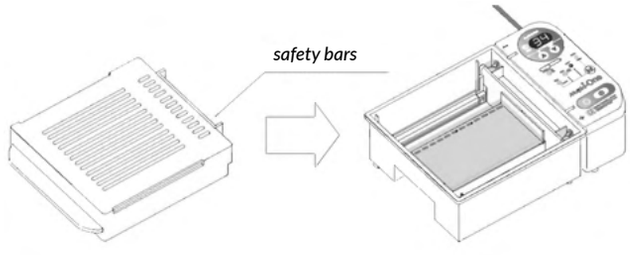


3.5(2) Sample application with MIDORI^{Green} Direct

- Mix samples and DNA markers with MIDORI^{Green} Direct stain at 1:10 (dye : samples) dilution rate
- If you expect only 1 or few DNA bands in a lane do not use more than 0.5 µl MIDORI^{Green} Direct, even if the sample volume is >5 µl
- For DNA Markers or samples with many bands use 1 µl of Midori Green
- Apply the desired amount of sample into the sample wells with a pipette.
- Approximately 12 µl of sample can be loaded into each 6 mm wide well, and approximately 4 µl can be loaded into each 2 mm wide well.
- Add ~5 µl of a DNA Marker (e.g. FastGene® DNA Marker: MWD50 / MWD100 / MWDP1) with added MIDORI^{Green} Direct.

3.6(1) Electrophoresis

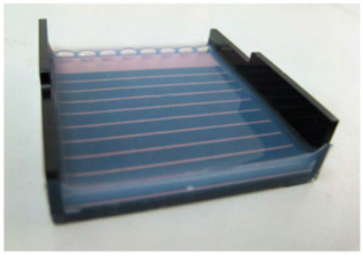
- Put the safety lid on top of the electrophoresis cell. Make sure that the safety bars fit correctly into the slits of the power supply.



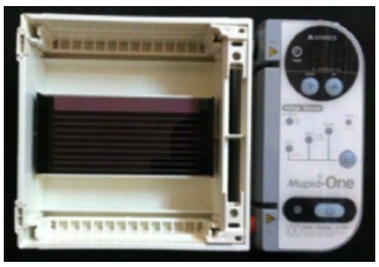
- Press the Run/Pause/Stop button to start the electrophoresis run.
- The red LED will light up, indicating that the system power is on.
- Check that air bubbles can be observed from the electrodes.
- When the time set on the timer is up, the power automatically turns off and an alarm sounds to indicate that the electrophoresis is complete. If continuous mode is set, press the Run/Pause/Stop button to end the run.

3.6(2) (Optional) Electrophoresis with MUPID™-ONE Illuminator

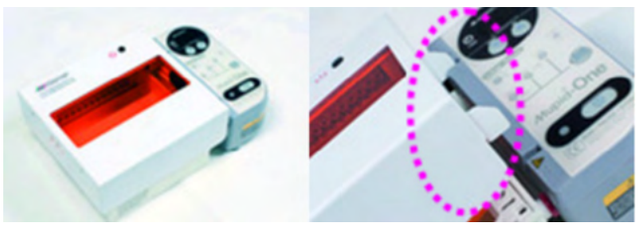
- The MUPID™-ONE Illuminator can be used to visualize the migration of the DNA and the progress of electrophoresis during the run.
- Use the NGE black tray to make an agarose gel and perform all the necessary steps for electrophoresis preparation (steps 1. - 3.5).



- Put the gel with the black gel tray in to the electrophoresis tank and apply samples and marker.



- Put the Illuminator lid on top of the electrophoresis tank. Make sure that the safety bars fit correctly into the slits of the power supply.



- Plug the MUPID™-ONE Illuminator via the power cable into a wall outlet.
- Press the Run/Pause/Stop button on the MUPID™-ONE to start the electrophoresis run.
- For viewing the gel, press the on/off button of the MUPID™-ONE Illuminator. This will activate the blue excitation light in the lid, that is used for detecting the DNA bands through the orange amber filter.

4. DNA detection

- When using MIDORI^{Green} Xtra, MIDORI^{Green} Direct or MIDORI^{Green} Easy, the best results can be achieved using the NGE exclusive Blue/Green LED light technology, our safe and very powerful technology for DNA detection.
- Use a transilluminator or gel documentation system with UV light when detecting nucleic acid stained with MIDORI^{Green} Advance.

NOTES

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