
Instruction Manual

PerfectBlue™

Horizontal Wide-Format Minigelsystems

Mini ExM & Mini ExW



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WARRANTY

PEQLAB guarantees that the horizontal electrophoresis system you have received has been thoroughly tested and meets its published specification.

However, immediately upon arrival, please check carefully that the shipment is complete and has not been damaged in transit. For missing parts or to report any kind of damage, please contact PEQLAB (see 'TECHNICAL SUPPORT AND ORDERING INFORMATIONS'). Please retain all packaging materials until the delivery has been completely checked since this will speed up the return of goods if required and reduce environmental impact. Any form of returns, replacements or credit notes must be agreed in advance by PEQLAB.

For the complete range of PerfectBlue™ electrophoresis and blotting systems, PEQLAB guarantees a warranty period of 36 months if the products have been used solely according to the instruction manual and if not agreed differently. After the warranty period has expired PEQLAB can offer repairs at low costs. No liability is accepted for loss or damage arising from incorrect use. PEQLAB's liability is limited to the repair or replacement of the unit or refund of the purchase price, at PEQLAB's discretion. PEQLAB is not liable for any consequential damages.

PEQLAB reserves the right to alter the technical specifications of the PerfectBlue™ electrophoresis or blotting systems without prior notice. This will enable us to implement developments as soon as they arise.

PACKAGING LIST

Unless differently agreed or marked on the delivery note the following items are included in shipment for the models PerfectBlue™ Mini ExM or Mini ExW:

- one buffer chamber with corrosion-protected platinum electrodes
- one safety lid with attached power cords
- one UV-transmissible gel tray with gaskets
- one casting chamber
- Mini ExM: 4 standard combs (1.5 mm thick, 2x 30 and 2 x 40 teeth)
- Mini ExW: 4 microtiter combs (1.5 mm thick, 50 teeth each)
- User Manual

SAFETY PRECAUTIONS

- Please, read this Instruction Manual carefully before using the gel system.
- Only use a CE marked DC power supply.
- Always disconnect the gel system from the power supply before adding electrophoresis buffer.
- Always disconnect the gel system from the power supply when it is not in use or before moving it.
- Running conditions for this unit should not exceed the maximum operating voltage or current.
- Do not fill the chamber with running buffer above the maximum fill line.

SYSTEM OVERVIEW

The PerfectBlue™ Horizontal Wide-Format Minigelsystems ExM and ExW allow the user to quickly cast and run up to 200 samples in a mini gel format; saving time and conserving lab space and reagents. Designed for high resolution at fast speeds, this system provides flat, even banding patterns and consistent results. The system features the leak-free gasketed UV gel tray and a separate casting chamber. The gasketed tray fits snugly into the caster eliminating the need to tape or seal the tray before pouring gels. Following the gel run, the tray can be placed back in the caster for convenient transport to the darkroom. The UV transmissible gel tray allows viewing and photography of stained bands with a UV light source very easily.

The PerfectBlue™ Horizontal Wide-Format Minigelsystems include a UV-transmissible gel tray, which has 2 (Mini ExM) or 4 (Mini ExW) comb positions, allowing the user to run 2, 3 or 4 series of samples equal distances simultaneously. All combs for the Mini ExW are designed in microtiter format for speedy sample loading utilizing a multichannel pipette in 1x or 2x format for maximum sample capacity per gel run. PEQLAB offers a wide variety of combs. For the Mini ExM model a microtiter comb in two different thicknesses is additionally available. Wall combs are available for the Mini ExW System, that allow you to run a shorter gel in a standard gel tray. Additionally, stand-alone casting chambers or the adjustable casting chamber JustCast are available for pouring gels while the chamber is in use.

For detailed information on available accessories visit www.peqlab.de or see 'TECHNICAL SUPPORT AND ORDERING INFORMATIONS'.

Technical Properties

PerfectBlue™	Cat. No.	Gel size (W x L)	Buffer volume	Voltage	Current	Time required
Mini ExM	40-1410	14.4 x 10.2 cm	600 ml	20-150 V	0-75 mA	30-60 min
Mini ExW	40-2314	23.0 x 14.0 cm	800 ml	20-150 V	0-75 mA	30-60 min

GENERAL INSTRUCTIONS

Setting up the system and pouring the agarose gel

1. Remove the lid from the gel chamber by holding the front of the buffer chamber with one hand and pulling the lid off by holding the center of the back of the lid. The cover is attached to the back of the unit at the connection of the power cords to the banana plugs.
2. For shipping and convenient storage, the gel tray is packaged inside the casting chamber.
3. To cast a gel, place the gel tray into the casting chamber making sure the tray is pushed all the way down and rests centered and level in the chamber. For best results, be sure to cast on a level surface. Levelling platforms are available if required. In order to facilitate pushing in or pulling out the gel tray from the casting chamber the rubber gaskets of the gel tray can get moistened. If using the adjustable casting chamber JustCast, the rubber gaskets can get removed from the gel tray.
4. When preparing the gel use electrophoresis-grade agarose and compatible electrophoresis buffer. The kind of agarose, its percentage as well as the buffer used is dependent on the properties of the target molecule and on the down-stream applications planned. (see 'REQUIRED REAGENTS & RECIPES'). The gel may be prepared in various ways: The agarose and buffer are mixed and heated over a heat plate by stirring or in a microwave oven until the agarose is completely dissolved.
5. The prepared gel must then be cooled down to 60 °C before casting to avoid warping the UVT gel tray due to excessive heat. If numerous gels are to be run in one day, a large volume of gel may be prepared and be placed in a covered bottle stored between 40-60 °C in a water bath or oven. This provides a ready gel supply in a warm liquid form that will solidify quickly when gels are cast.
6. Optionally: For casting smaller gels in order to conserve agarose, a wall comb (Model PerfectBlue™ Mini ExW only) may be used to divide the gel tray. Since wall combs do not contain any gaskets they should get sealed using 2% agarose before pouring the gel.
7. Pour or pipet the correct amount (see 'Agarose: gel volumes and percentage') of warm agarose (< 60 °C) onto the UVT gel tray that has been placed into the casting chamber. Immediately after pouring, insert the desired comb or combs into the comb slots to form the sample wells. Allow the gel to solidify completely. A single comb may be placed in either groove on the gel tray. If only a small portion of gel is required for proper sample separation, then 2 combs may be used. This also increases the number of samples per gel that may be run. Standard agarose should solidify completely in about 30 minutes at room temperature.
8. To remove the gel tray, hold the caster firmly with one hand, grasp the long side of the UVT gel tray and pull up slowly at an angle. Do not twist the gel tray relative to its transverse axis, since this might damage the gel tray at the grooves for the gaskets!

Loading of samples and electrophoresis

1. Once the gel is completely solidified, lift the tray out of the casting chamber as described above and place into the unit. The running position of the tray exposes the open ends of the agarose to the buffer.
2. Pour enough compatible running buffer into the unit to fill chamber and completely cover and submerge the gel with approx. 3 mm of buffer. A 'Fill Line' is located on each unit to clearly mark the maximal buffer level. See 'SYSTEM OVERVIEW/Technical properties' for approximate buffer volumes needed for your unit. Too little buffer may cause the gel to dry out during the run, while excess buffer will slow down DNA migration, increase the temperature in the gel and cause band distortion.
3. Carefully remove the comb(s) by tapping lightly to loosen, and slowly lift straight up out of the gel tray to avoid damage to the wells.
4. Load prepared samples into the wells. Samples should be mixed with a sample loading buffer; giving weight to the samples so that they drop evenly into the wells and contain tracking dyes to monitor the gel run. Refer to 'TECHNICAL SUPPORT AND ORDERING INFORMATIONS' for approximate well volumes. The combs supplied with the PerfectBlue Horizontal Wide Mini System ExW units are designed in a microtiter format. This format allows speedy sample loading using a multichannel pipet. The 25 or 26 tooth comb is in the 1x microtiter format and matches each tip of the multichannel pipet, while the 50 tooth comb is in the 2x format, loading every other lane.

NOTE: It is wise to always run a sample lane of a known 'molecular weight marker' to determine concentration and size of separated fragments after the gel run, and to aid in photo documentation and analysis.

5. Carefully slide the lid with attached power cords onto the unit. Then connect the power cords (4mm, male) to an appropriate DC power supply. Take care of a proper orientation of the electrical field. Remember that nucleic acids are negatively charged in an alkaline to neutral surrounding and therefore will migrate to the positively charged anode. In general the colour-code for positively charged electrodes is 'red'.
6. Turn on the power supply and run the gel at the appropriate electrical conditions (see 'System Overview/Technical properties'). Carefully monitor the gel run to avoid samples running into the path of another set of samples. In 0.5x TBE gels bromophenol blue comigrates with 300 bp DNA fragments and xylene cyanol approximately with 4 kbp DNA fragments.

Visualisation

When the gel run is completed and the tracking dye has migrated as far through the gel as desired or to the end of the gel, turn off the power supply and slide off the lid to disconnect from the power source. Carefully remove the tray containing the gel (wear gloves if ethidium bromide is present). The UV-transmissible gel tray makes for simple visualisation and photography with a UV light source without the need to remove the gel from the tray. The gel tray may be placed back into the casting chamber for convenient transport to the darkroom and to avoid damage to the gel.

Cleaning

The buffer chamber and tray should be rinsed under warm running water after each use. Use a mild detergent to get rid of any debris. It is recommended to allow the chamber to air dry rather than drying with a towel to avoid damage to the electrode wires.

Do not use ethanol or other organic solvents to clean acrylic products, because organic solvents cause acrylic to 'craze' or crack!

REQUIRED REAGENTS & RECIPES

Electrophoresis buffers

In general, electrophoresis buffers supply the ions necessary for electrophoresis and establishing a certain pH value in which the target molecule adapts to its the required electric charge. Nucleic acids for example will be negatively charged in an alkaline to neutral surrounding. Additionally, electrophoresis buffers often contain reagents which protect the target molecule from degradation (e.g. EDTA, which complexes bivalent cations and therefore inhibits DNases). If electrophoresis under denaturing conditions is desired (like for the electrophoresis of RNA), electrophoresis buffers will additionally contain reagents that eliminate the formation of secondary structures.

Below, you will find recipes for TAE and TBE, two of the most commonly used buffers for the electrophoresis of DNA. If the intention is to eventually isolate DNA from the gel, TAE buffer should be chosen. In comparison to TBE, migration will be faster and a better resolution of supercoiled DNA will be achieved when using TAE. However, because of TAE's limited buffering capacity, TBE should be selected for performing extended electrophoresis separations and if the electrophoresis chamber does not possess a system for buffer recirculation. PEQLAB's PerfectBlue 'Revolution' Systems are equipped with such an internal buffer recirculation system which effectively prevents the formation of pH and ion gradients during extended runs. Since agarose tends to create finer pore sizes and a more solid matrix in TBE, diffusion of DNA will be reduced and a more discrete band pattern will be achieved.

TAE (Tris-Acetate-EDTA) Buffer

1x working solution: 40 mM Tris-acetate, 1 mM EDTA

50x stock solution (1 l):
 242 g Tris-Base
 57.1 ml Glacial acetic acid
 100 ml 0.5 M EDTA (pH 8.0)
 made up to 1 l using H₂O

TBE (Tris-Borate-EDTA) Buffer

0.5 x working solution*: 45 mM Tris-Borat, 1 mM EDTA

5x stock solution (1 l)**:
 54 g Tris-Base
 27.5 g Boric acid
 20 ml 0.5 M EDTA (pH 8.0)
 made up to 1 l using H₂O

* 0.5x TBE is sufficient for agarose gel electrophoresis. For vertical electrophoresis in polyacrylamide gels, 1x TBE is often applied due to the comparatively smaller buffer reservoirs of vertical electrophoresis chambers.

** 5x TBE stock solutions tend to precipitate during long storage periods and should get remade. Because of this property, higher concentrations of TBE stock solutions should be avoided.

Agarose: Gel volumes and percentage

PEQLAB offers an extensive range of high quality agaroses, for many specific applications (see 'TECHNICAL SUPPORT AND ORDERING INFORMATIONS').

The required volume of the gel is calculated using the following formula.

$$\text{gel width (cm)} \times \text{gel length (cm)} \times \text{gel thickness (cm)} = \text{required volume agarose solution (ml)}$$

The following volumes will result:

Model	Gel size (cm)	Gel thickness (cm)			
		0.25	0.5	0.75	1.0
PerfectBlue™ Mini ExM	14 x 10 (B x L)	35 ml	70 ml	105 ml	140 ml
PerfectBlue™ Mini ExW	23 x 14 (B x L)	81 ml	161 ml	242 ml	322 ml

The optimal range of DNA fragment sizes separated by any electrophoresis experiment is dependent on the agarose concentration of the gel. The higher the agarose concentration, the better small fragments are separated from each other and vice versa. However, for the smallest or largest fragment lengths, the usage of specialized agaroses or polyacrylamide gels should be considered (see table below), since a 3% agarose solution solidifies rapidly and a 0.3% agarose gel is very soft and difficult to handle.

Agarose content (w/v)	Agarose (g)	Puffer (ml)	optimal separation range (kb)
0.3%	0.3	100	5-30
0.5%	0.5	100	1-15
0.7%	0.7	100	0.8-10
1.0%	1.0	100	0.5-7
1.2%	1.2	100	0.3-6
1.5%	1.5	100	0.2-4
2.0%	2.0	100	0.1-3
3.0%	3.0	100	<0.1

Ethidium bromide

The gel may be stained during or following the run with a variety of stains for photodocumentation. The most common stain for DNA is ethidium bromide. Because of its capacity to intercalate between the bases of a nucleic acid strand and altering the sterical properties of DNA, ethidium bromide is judged to be highly mutagenic. Therefore appropriate safety measures must be applied.

Ethidium bromide may be added directly to the gel before pouring it at a concentration of 0.1 to 0.5 µg/ml. However, being positively charged, ethidium bromide will migrate to the cathode during the electrophoresis leading to non-homogeneous staining. Improved results can be obtained by incubating the gel after the electrophoresis is finished in electrophoresis buffer containing 0.5 µg/ml ethidium bromide for 5 to 20 min. Subsequently the gel should get rinsed in electrophoresis buffer without ethidium bromide for up to 20 min in order to reduce background signal.

Loading buffer/Sample buffer

Samples are prepared and mixed with loading buffer before applying to the prepared gel. Sample buffers contain dyes for visibility and glycerol to provide weight to the samples. This increased sample density ensures samples load evenly into the wells and do not float out during loading. Dyes also migrate toward the anode end of the electrophoresis chamber at predictable rates allowing the gel run to be monitored. In 0.5x TBE gels bromophenol blue migrates at the same rate as 300 bp DNA fragments and xylene cyanol approximately at the same rate as 4 kbp DNA fragments.

6x DNA sample buffer: 0.25 % (w/v) bromophenol blue
 0.25 % (w/v) xylene cyanol FF
 30 % (v/v) glycerol

Molecular weight marker

Markers are run on each gel to monitor the quality of sample separation and to enable a size estimation of specific bands. By running a known marker of a specific concentration in parallel, the DNA amount of the unknown samples can be estimated. PEQLAB offers an extensive range of DNA and RNA markers. For detailed information please contact us or visit www.peqlab.de.

TROUBLESHOOTING

Some possible solutions to potential problems are listed below. If these suggestions are unclear or unsuccessful, please contact PEQLAB.

Problem: Agarose leaks into casting chamber when pouring gel

Check to see if the gasket is firmly seated in the grooves on the ends of the UVT gel tray. Reseat gasket if necessary by removing and rinsing under warm running water, then reseat evenly in the tray groove.

Problem: Bands seem to be running at an angle (Gel smiling).

Check to be sure the casting is being done on a level surface. Also confirm that the gel tray is inserted all the way into the unit and rests on the platform for level gel casting. The voltage may be too high. Try lowering the voltage setting on the power supply.

Problem: Samples seem to be running unevenly in certain areas.

Check that the platinum electrode wire is intact and running evenly across the base of the chamber and up the side to the junction of the banana plug. If there appears to be a break in the electrode connection contact PEQLAB immediately. This problem may also be caused by regularly casting with very hot agarose gel (>60 °C). Always cool the melted agarose to below 60 °C before casting to avoid warping the UVT gel tray. Warping the gel tray will cause all subsequent gels to be cast unevenly.

Problem: Samples do not band sharply and appear diffuse in the gel.

Gels should be no more than 5 mm thick and be allowed to solidify completely before running. Standard agarose should solidify in about 30 minutes. If low melting point agarose is used, it may be necessary to completely solidify gels at a cooler temperature in the refrigerator or cold room. Gels should be submerged in 3-5 mm of buffer to avoid gel dry out, but excess buffer (>5 mm) can cause decreased DNA mobility and band distortion.

Problem: Samples are not moving as expected through the gel, remaining in the wells, running 'backwards' or diffusing into the gel.

Check to be sure that a complete power circuit is achieved between the unit and the power supply. Platinum wire and banana plugs should be intact. To test, simply fill the unit with running buffer and attach to the power supply without a gel or gel tray in the unit. The platinum wires on both sides of the unit should produce small bubbles as the current passes through. If a complete circuit does not exist there will be little to no bubbles. If samples appear to run backwards through the gel or there are no bands visible, check to be sure that the gel tray was placed in the electrophoresis chamber in the proper orientation. If the orientation or polarity is reversed, the samples will run backwards or migrate off the gel. The tray should be placed in the chamber with the comb at the edge of the tray closest to the cathode side of the chamber.

Problem: When the comb is removed from the gel the sample well is ripped and damaged.

Always make sure to allow the gel to solidify completely before moving the tray, unit, or removing the comb. To avoid damage to the sample wells, gently rock the comb back and forth lightly to loosen, and then slowly pull the comb straight up out of the gel tray. This rocking helps to avoid suction as the comb is removed. Alternatively, once casting is complete and the gel tray is placed in the running orientation, simply submerge the gel in running buffer to help loosen the comb.

Problem: The gel seems to run slower under the usual running conditions.

The volume of running buffer used to submerge the gel should only be between 3-5 mm over the gel surface. Gel should be completely submerged to avoid the gel from drying out, which can smear the bands and possibly melt the gel due to overheating. If excessive running buffer is added the mobility of the DNA decreases and band distortion may result. Excess buffer causes heat to build up and buffer condensation inside the unit may result.

TECHNICAL SUPPORT AND ORDERING INFORMATION

For technical questions please contact us by phone (+49 (0)9131 610 7020) or e-mail (info@peqlab.de). Please find detailed information on PEQLAB's products on www.peqlab.de.

PerfectBlue™ Mini ExM

Item	Description	Cat. No.
Gel system Mini ExM	complete system for gels 14 x 10 cm (W x L)	40-1410
Casting chamber	Casting chamber for up to 2 gel trays	40-1410-CST
Gel tray	UV-transmissible gel tray and gaskets	40-1410-UVT
MultiCast Casting chamber	Casting chamber and 2 UV-transmissible gel trays	40-1410-MC
Gaskets	2 rubber gaskets for gel tray	40-1410-GK
Standard combs	1.5 mm 10 teeth 68 µl*	40-1410-10D
	1.5 mm 20 teeth 29 µl*	40-1410-20D
	1.5 mm 30 teeth 14 µl*	40-1410-30D
	1.5 mm 40 teeth 10 µl*	40-1410-40D
	1.0 mm 10 teeth 46 µl*	40-1410-10C
	1.0 mm 20 teeth 19 µl*	40-1410-20C
	1.0 mm 30 teeth 9 µl*	40-1410-30C
	1.0 mm 40 teeth 6 µl*	40-1410-40C
Microtiter combs	1.5 mm 15 teeth 40 µl*	40-1410-MTD
	1.0 mm 15 teeth 27 µl*	40-1410-MTC

* volumes are calculated for a gel thickness of 5 mm

PerfectBlue™ Mini ExW

Item	Description	Cat. No.
Gelsystem Mini ExW	complete system for gels 23 x 14 cm (W x L)	40-2314
Casting chamber	Casting chamber for sealing a gel tray	40-2314-CST
Gel tray	UV-transmissible gel tray and gaskets	40-2314-UVT
Gaskets	2 rubber gaskets for gel tray	40-2314-GK
Wall comb	Wall comb for dividing up the gel tray	40-2314-WC
Microtiter combs	1.5 mm 25 teeth 40 µl*	40-2314-MTD
	1.5 mm 26 teeth 40 µl*	40-2314-26D
	1.5 mm 50 teeth 15 µl*	40-2314-MT2D
	1.0 mm 25 teeth 27 µl*	40-2314-MTC
	1.0 mm 26 teeth 27 µl*	40-2314-26C
	1.0 mm 50 teeth 10 µl*	40-2314-MT2C

* volumes are calculated for a gel thickness of 5 mm

JustCast adjustable casting chamber

For the simple, leak-proof casting of up to three Mini S gels, two Mini M gels, two Mini L gels, one Mini ExM gel or one Mini ExW gel.

Item	Description	Cat. No.
JustCast	Adjustable Casting Chamber for PerfectBlue™ Minigelsystems, including a 3-point levelling system with water level	40-CST

Power Supplies

Do not hesitate to contact us for advice on which Power Supply is most suitable for your application.

Item	Ports	max. Voltage (V)	max. Current (mA)	Power (W)	Cat. No.
EV222	3	200	200	20	55-EV222
EV243	3	400	300	50	55-EV243
EV231	4	300	1000	150	55-EV231
EV265	4	600	500	150	55-EV265
EV202	4	300	2000	300	55-EV202
EV261	4	600	1000	300	55-EV261
EV215	4	1200	500	300	55-EV215
EV232	4	3000	150	150	55-EV232
EV233	4	3000	300	300	55-EV233
EV262	4	6000	150	300	55-EV262

Agaroses

Item	Purpose	Amount	Cat. No.
peqGOLD Universal-Agarose	Suitable for standard applications. Separation range between 0.05 and 50 kb.	100 g	35-1010
		500 g	35-1020
		1000 g	35-1030
peqGOLD Universal-Agarose Tabs	Convenient tablet format. Suitable for standard applications. Separation range between 0.05 and 50 kb.	50 g	35-7010
		250 g	35-7020
		500 g	35-7030
peqGOLD 'Low Melt'-Agarose	For the preparative separation of DNA fragments between 0.08 and 20 kbp.	25 g	35-2010
		100 g	35-2020
		250 g	35-2030
peqGOLD MoSieve-Agarose MS-500	Especially for high-resolution separation of small fragments (0.01 - 1 kbp).	25 g	35-3010
		100 g	35-3020
		250 g	35-3030
peqGOLD MoSieve-Agarose MS-1000	Especially for high-resolution separation of small fragments between 0.05 - 2 kbp.	25 g	35-4010
		100 g	35-4020
		250 g	35-4030
peqGOLD MegaBase-Agarose	Especially for separation of larger DNA fragments between 0.2 and 50 kbp.	25 g	35-5010
		100 g	35-5020
		250 g	35-5030
peqGOLD 'Pulsed Field'-Agarose	Especially for 'Pulsed Field' applications.	25 g	35-6010
		100 g	35-6020
		250 g	35-6030

LITERATURE

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