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Instruction Manual

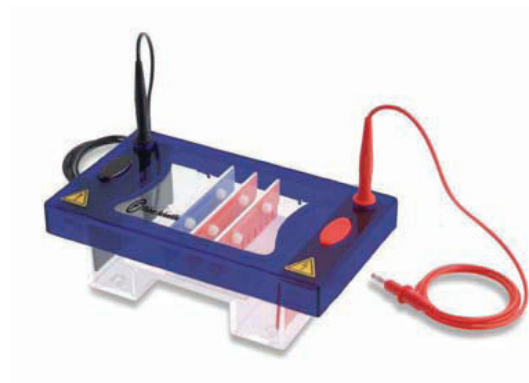
Horizontal Electrophoresis Units, SUB-GEL

- 11863303**
SUB-GEL Mini
- 11853303**
SUB-GEL Midi
- 11833293**
SUB-GEL Midi-Plus
- 11843303**
SUB-GEL Maxi

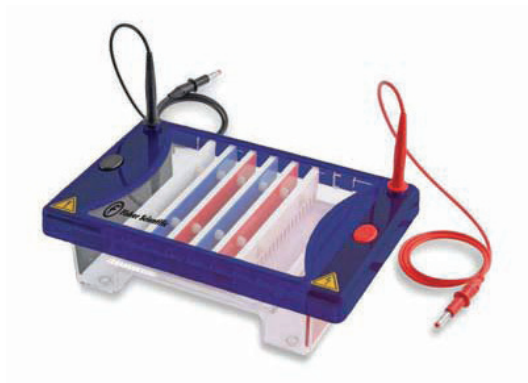
Horizontal Electrophoresis Units SUB-GEL



11863303 SUB-GEL Mini



11853303 SUB-GEL Midi



11833293 SUB-GEL Midi-Plus



11843303 SUB-GEL Maxi



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Safety Instructions

- When used correctly, these units pose no health risk
- However, these units can deliver dangerous levels of electricity and are to be operated only by qualified personnel following the guidelines laid out in this instruction manual
- Anyone intending to use this equipment should read the complete manual thoroughly
- The unit must never be used without the safety lid correctly in position
- The unit should not be used if there is any sign of damage to the external tank or lid
- These units comply with the statutory CE safety directives:
 - 73/23/EEC: Low Voltage Directive: IEC 1010-1:1990 plus Amendment 1:1992
 - EN 61010-1:1993/BS EN 61010-1:1993



Packing List

SUB-GEL Mini

Cat. No	Gel Casting Tray	Tray Dams	Comb Thickness Teeth No. & Qty	Loading Guides	Cables
11863303	11847573 70mm x 70mm (MS7-UV7)	11837633 (MS7-UVDAM) x2	11827563 1mm, 8 sample (MS7-8-1) x2	11827563 Strips (MS7-LG)	1 Red/1 Black
	11837573 70mm x 100mm (MS7-UV10)			11867573 Viewing platform adhesive (MS7-WP)	

SUB-GEL Midi

Cat. No	Gel Casting Tray	Tray Dams	Comb Thickness Teeth No. & Qty	Loading Guides	Cables
11853303	11873353 100mm x 70mm (MS10-UV7)	11807633 (MS10-UVDAM) x 2	11813323 1mm, 16 sample (MS10-16-1) x 2	11823353 Strips (MS10-LG)	1 Red/1 Black
	11863353 100mm x 100mm (MS10-UV10)			11803363 Viewing platform adhesive (MS10-WP)	

SUB-GEL Midi-Plus

Cat. No	Gel Casting Tray	Tray Dams	Comb Thickness Teeth No. & Qty	Loading Guides	Cables
11833293	11803423 150mm x 70mm (MS15-UV7)	11817633 (MS15-UVDAM) x 2	11823393 1mm, 20 sample (MS15-20-1) x2	11843413 Strips (MS15-LG)	1 Red/1 Black
	11883413 150mm x 100mm (MS15-UV10)			11823423 Viewing platform adhesive (MS15-WP)	
	11893413 150mm x 150mm (MS15-UV15)				

SUB-GEL Maxi

Cat. No	Tray	Tray Dams	Comb Thickness Teeth No. & Qty	Loading Guides	Cables
11843303	11813473 200mm x 100mm (MS20-UV10)	11827633 (MS20-UVDAM) x 2	11873433 1mm, 20 sample (MS20-20MC-1)x2	11873463 Strips (MS20-LG)	1 Red/1 Black
	11823473 200mm x 200mm (MS20-UV20)			11853473 Viewing platform adhesive (MS20-WP)	

The packing lists should be referred to as soon as the units are received to ensure that all components have been included. The unit should be checked for damage when received. Please contact your nearest Fisher Scientific supplier if there are any problems or missing items.

System Details

Construction

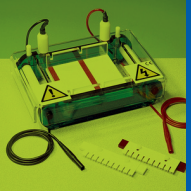
- Rugged acrylic construction
- All acrylic joints chemically bonded
- Doubly insulated cables, rated safe up to 1,000 volts
- Gold plated electrical connectors, corrosion free and rated safe up to 1,000 volts
- Recessed power connectors, integral with the safety lid
- 0.2mm diameter platinum electrodes, 99.99% pure
- Removable UV transparent gel casting trays
- Combs, colour coded for thickness:
 - 1.0mm - White
 - 1.5mm - Red
 - 2.0mm - Blue
- Combs, adjustable in height

Usage Guidance and Restrictions

- Maximum altitude 2,000m
- Temperature range between 4°C and 65°C
- Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C
- Not for outdoor use

This apparatus is rated POLLUTION DEGREE 2 in accordance with IEC 664.

POLLUTION DEGREE 2, states that: 'Normally only non-conductive pollution occurs occasionally, however, a temporary conductivity caused by condensation must be expected'.



Care and Maintenance

Cleaning SUB-GEL Units

Units should be cleaned using warm water and a mild detergent. **Water at temperatures above 60°C can cause damage to the unit and components.**

The tank should be thoroughly rinsed with warm water or distilled water to prevent build up of salts, but care should be taken not to damage the enclosed electrode and vigorous cleaning is not necessary or advised.

Air drying is preferable before use.

The units should only be cleaned with the following:

- Warm water with a mild concentration of soap or other mild detergent, the units should not be left in detergents for more than 30 minutes
- They should be then rinsed with distilled water immediately afterwards
- Compatible detergents include dishwashing liquid, hexane and aliphatic hydrocarbons

The units should never come into contact with the following cleaning agents, these will cause irreversible and accumulative damage:

- Acetone, Phenol, Chloroform, Carbon tetrachloride, Methanol, Ethanol, Isopropyl alcohol, Alkalis

RNase Decontamination

This can be performed using the following protocol:

- Clean the units with a mild detergent as described above
- Wash with 3% hydrogen peroxide (H₂O₂) for 10 minutes
- Rinse with 0.1% DEPC (diethyl pyrocarbonate) treated distilled water (Cat. No 10245203, refer to page 17)

Caution

DEPC is a suspected carcinogen. Always take the necessary precautions when using.

Using the SUB-GEL Units

Setting Up the SUB-GEL Units

Instructions for Fitting Electrode Cables

- Note the position of the lid on the unit. This shows the correct polarity and the correct orientation of the cables, black is negative and red positive
- Remove the lid from the unit. Note, if the lid is not removed, fitting the cables may result in untightening of the gold plug and damage to the electrode
- Screw the cables into the tapped holes as fully as possible so that there is no gap between the lid and the leading edge of the cable fitting
- Refit the lid

Instructions for fitting Loading Guides (optional)

These can be fitted to enhance visibility of the wells if desired. They can be fitted to the white vinyl platform sheet or to the unit itself.

- Seat the tray in the unit and note the position of the comb grooves. The samples run black to red but the trays can be used frontward or backwards so ensure that the comb grooves closest to the black electrode are marked
- Remove the tray
- Peel the back off of the loading guide and carefully apply the loading guide directly to the gel platform

The unit is now ready to be used.

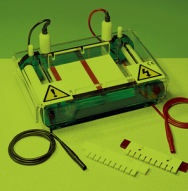
Gel Preparation

- The following table shows the volume of agarose solution required to make the desired agarose gel for each unit tray size. For a standard 0.7% agarose gel, add 0.7 grammes of agarose to 100mL of 1X TAE or TBE solution. The same 1X solution should be used in the tank buffer solution

SUB-GEL Mini		SUB-GEL Midi		SUB-GEL Midi-Plus		SUB-GEL Maxi	
Tray	Gel volume for a 5mm thick gel	Tray	Gel volume for a 5mm thick gel	Tray	Gel volume for a 5mm thick gel	Tray	Gel volume for a 5mm thick gel
70mm x 70mm	25mL	70mm x 100mm	35mL	70mm x 150mm	52.5mL	100mm x 200mm	100mL
100mm x 70mm	35mL	100mm x 100mm	50mL	100mm x 150mm	75mL	200mm x 200mm	200mL
				150mm x 150mm	112.5mL		

- Add the agarose powder to a conical flask
- Add the appropriate amount of 1X TAE or TBE solution from the table above. To prevent evaporation during the dissolving steps below, the conical flask should be covered with parafilm
- Dissolve the agarose powder by heating the agarose either on a magnetic hot plate with stirring bar or in a microwave oven. If using the microwave method, the microwave should be set at around a 400 watt or medium setting and the flask swirled every minute. The solution should be heated until all crystals are dissolved. This is best viewed against a light background. Crystals appear as translucent crystals. These will interfere with sample migration if not completely dissolved

The gel must be cooled to between 50°C and 60°C before pouring.



Gel Pouring

Using trays with Casting Dams

- Fit the casting dams over each end of the tray and place onto a level surface. The dams should be fitted so that there is no gap between the sides of the tray and the groove in the dams. This will ensure that there is no possibility of gel leakage (Fig.1)
- Place the comb(s) in the grooves. Each tray has more than one comb groove so that multiple combs can be used (Fig.2). Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells
- Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip
- Allow the agarose to set, ensuring that the gel remains undisturbed (Fig.3)
- Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank

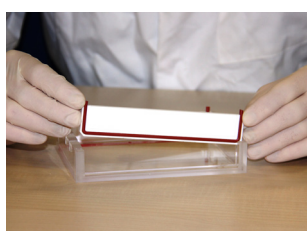


Fig.1

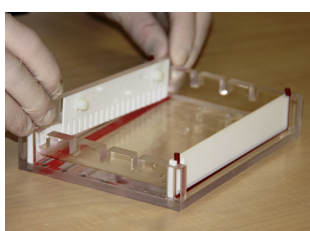


Fig.2

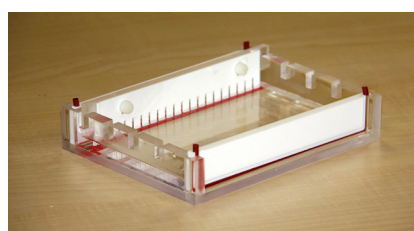


Fig.3

Using Traditional tape method:-

- Autoclave or plastic backed general tape should be used. A length 5cm longer than the width of each end of the tray should be cut. One length should be placed over one end of the tray and stuck 1cm in from the tray edge. This should then be folded and the edges sealed securely. Repeat for the other end and place onto a level surface for gel pouring
- Place the comb(s) in the grooves. Each tray has more than one comb groove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells
- Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipettor tip
- Allow the agarose to set, ensuring that the gel remains undisturbed
- Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank

Running the Gel

- Samples should be mixed with a suitable marker dye or loading buffer before loading to allow the sample to sink into the well, and aid visualisation of how far the samples have migrated during electrophoresis. Consult your laboratory manual for details on loading buffers, also refer to page 17 for list of Gel Loading Agents
- Load the samples into the wells taking care not to damage the sides or bottoms of the wells. Replace the lid correctly BEFORE connecting the leads to the power supply. Suggested power supplies include Cat. No 12643546 (twin output) and Cat. No 12613546 (four output)



Cat. No 12643546



Cat. No 12613546

- Set the voltage and current to suit the electrophoretic application. As a guide, to obtain the optimum resolution of DNA fragments, agarose gels should not be run at a field strength greater than 5V/cm
- IMPORTANT: Do not exceed the recommended voltage or current as this may result in poor band resolution and may result in damage to the unit

At the End of the Run

- Turn the power supply settings to zero, turn off mains supply and disconnect the power leads
- Visualise the run progression or final separation on a UV transilluminator
- At the end of the run rinse the apparatus with DISTILLED WATER ONLY
- IMPORTANT: Acrylic plastic is NOT resistant to aromatic or halogenated hydrocarbons, ketones, esters, alcohols (over 25%) and acids (over 25%). These will cause 'crazing' especially of the UV transparent plastic and should NOT be used for cleaning. DO NOT use abrasive creams or scourers. Dry components with clean tissues prior to use
- Ensure that the connectors are clean and dry before usage or storage



Preparation of Agarose Gels

The amount of agarose needed for a particular % gel needs to be calculated. For example, an 0.8% gel is made by dissolving 0.8g of agarose powder in 100mL running buffer. The running buffer composition of the gel should be identical to that used in the buffer tank. The agarose needs to be fully dissolved before the gel can properly form. This can be achieved by heating in a swirling water bath or incubator set to 70°C, or by heating on a magnetic heating block with a magnetic stirring bar inserted. The flask should always be covered to prevent evaporation of the buffer and a higher concentration gel resulting. These methods may take longer than 1 hour for the agarose to fully dissolve. Alternatively, the agarose can be dissolved in about 5 to 10 minutes using a microwave oven. The agarose solution should be covered and the microwave set to low. The agarose dissolves better if the microwave is periodically stopped and the solution swirled. Before pouring, the agarose solution should be checked for undissolved agarose crystals which can affect the mobility characteristics of gels. If these are present, agarose dissolving should be continued. The agarose solution should be cooled to 50°C to 60°C before pouring. If poured at too hot a temperature, the gel will be more likely to leak and the comb may become distorted.

Agarose Gel Electrophoresis

DNA mobility: DNA fragments as small as 1kb or less can be separated using agarose gel electrophoresis. For fragments smaller than 0.1kb, polyacrylamide gels are more suited.

RNA mobility: Either before or during electrophoresis, RNA should be denatured. For example:

- RNA fragments which have denatured with glyoxal and dimethyl sulfoxide can be separated on neutral agarose gels, or
- RNA can be fractionated on agarose gels containing methylmercuric hydroxide or formaldehyde

RNA samples usually require longer runs or buffers that are easily depleted, so it is necessary to circulate the buffer. Northern analyses should not normally be run on a mini gel tank.

Separation Performance

Gel concentration, running buffer, voltage, temperature, conformation, and the presence of ethidium bromide may all affect separation results.

Gel Concentration Selection

The range of fragment sizes to be separated will determine the choice of agarose concentration for a gel. Typical agarose concentration is 0.5% to 3.0%. For large DNA fragments low percentage gels are required, while, for small DNA fragments, high percentage gels are recommended. Weak gels (<0.5% agarose) should be electrophoresed at low temperatures (e.g. -4°C). Agarose gels of 0.75% to 1.0%, for routine electrophoresis, are recommended for a wide range of separations (0.15 to 15kb). 2% to 4% agarose gels are usually selected for PCR fragment resolution.

If the gel has to be photographed, thin gels (2mm to 3mm) with low percentage agarose are better than thick or high percentage gels. The latter may produce increased opacity and autofluorescence.

The table below offers suggested agarose concentration for separating various fragment sizes. Additionally, resolution ranges can be extended by using special agaroses.

Agarose (%)	Effective Resolution of Linear DNA Fragments (kb)	
0.5	30 →	1.0
0.7	12 →	0.8
1.0	10 →	0.5
1.2	7 →	0.4
1.5	3 →	0.3
2.0	3 →	0.2
3.0	3 →	0.1

Electrophoresis Buffer Selection

TAE buffer provides optimal resolution of fragments >4kb in length, while for 0.1 to 3kb fragments, TBE buffer should be selected. TBE has a higher buffering capacity and lower conductivity than TAE and therefore should be used for high voltage electrophoresis. Additionally, TBE buffer generates less heat than TAE at an equivalent voltage and does not allow a significant pH drift. Refer to page 16 for list of Buffers for DNA Electrophoresis Applications.

Note: Because of its lower buffering capacity, TAE may need to be circulated or mixed from time to time for full length electrophoresis, especially at higher voltages.

Temperature

Electrophoresis at high voltages produces heat. Additionally, high conductivity buffers such as TAE generate more heat than low conductivity buffers. Care should be taken in agarose gel electrophoresis with voltages greater than 175V, as heat build-up may generate gel artifacts such as S-shaped migration fronts, and in extended electrophoresis runs may melt the agarose gel. Low melting point agarose gels should not be used for high voltage runs.

DNA Visualisation

To establish progress of double stranded DNA, ethidium bromide (0.5µg/mL) is often added to running buffer (refer to page 17 for list of Ethidium Bromide products). The dye's fluorescence properties allows the band to be visualised under a UV lamp. However, ethidium bromide may slow the DNA migration rate by approx 15%. As an alternative, after electrophoresis, the gel may be stained in an ethidium bromide solution (0.5µg/mL H₂O) for 15 to 60 minutes and then viewed or photographed on a UV transilluminator.

Note: Staining time should be minimised to prevent small nucleic acid fragments from diffusing out of the gel.

Background fluorescence of unbound ethidium bromide can be minimised through destaining by soaking the gel for 5 minutes in 0.01M MgCl₂, or for 30 minutes in deionised water.



CAUTION !

Ethidium bromide is a known mutagen. Always wear gloves when handling. Wear UV safety goggles and protect skin when using any UV light source.



APPENDIX

Combs Specifications

The following tables detail the full range of combs available for use with SUB-GEL Units.

The Use of Multiple Combs

All Fisher Scientific systems allow the use of multiple combs. This facility greatly increases the number of samples of 'mini-prep' plasmid DNAs that can be screened. By using the bottom row of wells on the gel, quantitative standards may be included for Southern blot hybridisation.

Note: Standards should be added to the bottom row and allowed to migrate into the gel for a few minutes before electrophoresis is complete.

11863303 - SUB-GEL Mini

Combs	Thickness 0.75mm		Thickness 1.0mm		Thickness 1.5mm		Thickness 2.0mm	
	Cat. No	Sample size, µL	Cat. No	Sample size, µL	Cat. No	Sample size, µL	Cat. No	Sample size, µL
Prep 1, Marker 1	11873473	152	11823483	203	11833483	304	11843483	405
Prep 2, Marker 2	11833493	68	11843493	90	11857553	135	11867553	180
Prep 4, Marker 2	11877553	36	11887553	48	11897553	72	11807563	96
8 sample, MC	11857563	8	11867563	11	11877563	17	11887563	23
8 sample	11817563	19	11827563	25	11837563	37	11847563	50
10 sample	11883473	14	11893473	18	11803483	27	11813483	36
12 sample, MC	11853483	10	11863483	14	11873483	20	11883483	27
16 sample	11893483	7	11803493	10	11813493	15	11823493	20

MC = multichannel pipettor compatible

11853303 - SUB-GEL Midi

Combs	Thickness 0.75mm		Thickness 1.0mm		Thickness 1.5mm		Thickness 2.0mm	
	Cat. No	Sample size, µL	Cat. No	Sample size, µL	Cat. No	Sample size, µL	Cat. No	Sample size, µL
Prep 1, Marker 1	11883303	270	11833313	360	11843313	540	11853313	720
Prep 2, Marker 2	11843323	118	11893323	158	11803333	236	11813333	315
Prep 4, Marker 2	11863333	57	11873333	77	11883333	115	11893333	153
8 sample	11803343	30	11813343	41	11823343	61	11813353	81
10 sample MC	11893303	20	11803313	27	11813313	41	11823313	54
12 sample	11863313	17	11873313	23	11883313	34	11893313	45
16 sample	11803323	12	11813323	16	11823323	24	11833323	32
20 sample MC	11853323	10	11863323	14	11873323	20	11883323	27
25 sample	11823333	7	11833333	10	11843333	15	11853333	20

MC = multichannel pipettor compatible

11833293 - SUB-GEL Midi-Plus

Combs	Thickness 0.75mm		Thickness 1.0mm		Thickness 1.5mm		Thickness 2.0mm	
	Cat. No	Sample size, µL	Cat. No	Sample size, µL	Cat. No	Sample size, µL	Cat. No	Sample size, µL
Prep 1, Marker 1	11823363	371	11813373	495	11823373	743	11833373	990
Prep 2, Marker 2	11803393	169	11853393	225	11863393	338	11873393	450
Prep 4, Marker 2	11803413	91	11813413	122	11823413	182	11833413	243
10 sample	11833363	34	11843363	45	11853363	68	11863363	90
10 sample, MC	11873363	22	11883363	29	11893363	44	11803373	59
12 sample	11843373	30	11853373	41	11863373	61	11873373	81
14 sample, MC	11883373	22	11893373	29	11803383	44	11813383	59
16 sample, MC	11823383	20	11833383	27	11843383	41	11853383	54
18 sample, MC	11863383	8	11873383	11	11883383	17	11893383	23
20 sample	11813393	16	11823393	21	11833393	32	11843393	43
28 sample, MC	11883393	8	11893393	11	11803403	17	11813403	23
30 sample, MC	11823403	9	11833403	13	11843403	19	11853403	25
35 sample	11863403	7	11873403	10	11883403	15	11893403	20

MC = multichannel pipettor compatible

11843303 - SUB-GEL Maxi

Combs	Thickness 0.75mm		Thickness 1.0mm		Thickness 1.5mm		Thickness 2.0mm	
	Cat. No	Sample size, μL	Cat. No	Sample size, μL	Cat. No	Sample size, μL	Cat. No	Sample size, μL
Prep 1, Marker 1	11833423	508	11883423	675	11893423	1,013	11803433	1,350
Prep 2, Marker 2	11853433	236	11803443	315	11813443	473	11823443	630
Prep 4, Marker 2	11853453	115	11803463	153	11813463	230	11823463	306
10 sample	11843423	54	11853423	72	11863423	108	11873423	144
16 sample	11813433	30	11823433	41	11833433	61	11843433	81
20 sample, MC	11863433	20	11873433	27	11883433	41	11893433	54
25 sample	11833443	16	11843443	21	11853443	32	11863443	42
30 sample	11873443	13	11883443	17	11893443	26	11803453	34
36 sample	11813453	11	11823453	14	11833453	22	11843453	29
40 sample, MC	11863453	8	11873453	11	11883453	17	11893453	23
50 sample	11833463	8	11843463	10	11853463	16	11863463	21

MC = multichannel pipettor compatible

SUB-GEL Midi 96 Combs

Cat. No	Description
11893343	SUB-GEL Midi 96 comb 8 sample MC + 1 marker, 1mm thick comb block
11853343	SUB-GEL Midi 96 comb 8 sample MC + 1 marker, 1.5mm thick comb block
11873343	SUB-GEL Midi 96 comb 8 sample MC + 2 marker, 1mm thick comb block
11833343	SUB-GEL Midi 96 comb 8 sample MC + 2 marker, 1.5mm thick comb block
11803353	SUB-GEL Midi 96 comb 8 sample MC, 1mm thick, one marker lane
11863343	SUB-GEL Midi 96 comb 8 sample MC, 1.5mm thick, one marker lane
11883343	SUB-GEL Midi 96 comb 8 sample MC, 1mm thick, two marker lanes
11843343	SUB-GEL Midi 96 comb 8 sample MC, 1.5mm thick, two marker lanes

MC = multichannel pipettor compatible



Fisher BioReagents[™]

Your source for high purity products for nucleic acid electrophoresis.

Packaging for Safety, Convenience and Product Quality

Fisher BioReagents come in a wide variety of innovative packaging designed for safety, environmental protection, convenient handling and storage, and preservation of product integrity. The primary container is included in the product description of most chemicals in this catalogue.

Primary containers include:

- Plastic and glass bottles, jars
- Specialised acid containers
- Square poly bottles
- Sterility proof sachets
- Poly pails
- Polypac[™] containers
- Compact, laminated boxes



Fisher BioReagents[™]: Purity Grades for Every Application

Material Grade	Definition
Certified	Reagent chemicals for which the purity standard is established by Fisher Chemical. Purity is guaranteed to meet published maximum limits of impurities.
DNA grade	Designates reagents suitable for use in molecular biology applications involving the manipulation of DNA. Tested for specific contaminants, such as DNase and protease.
DNA synthesis	Designates reagents suitable for use with automated DNA synthesis instrumentation.
Electrophoresis	Material used specifically for electrophoresis applications.
Genetic analysis grade	Material that is specially prepared for various molecular cloning applications. Tested for specific contaminants, such as DNase and RNase.
IEF grade	Material suitable for use with isoelectric focusing of proteins.
Islet isolation grade	Material suitable for isolation of pancreatic islets.
Molecular biology grade	Designates reagents suitable for use in molecular biology applications. Tested for specific contaminants, such as nucleases and bacteria, where appropriate.
Molecular genetics	Reagent chemicals that have been specifically purified and assayed for molecular genetics applications.
PCR grade	Material suitable for use in Polymerase Chain Reaction (PCR*).
Peptide synthesis	Designates reagents suitable for use with protein synthesis instrumentation.
Protein electrophoresis grade	Material used specifically for protein electrophoresis applications.
Sequencing	Material designed for use with automated DNA or protein sequencing equipment.
Super pure	Material with a purity level exceeding the various monograph grades.
Tissue culture grade	Materials of superior quality where there are no published standards and that are suitable for use in tissue culture applications.

*Polymerase Chain Reaction (PCR) is a process covered by patents owned by Hoffmann-La-Roche



Fisher BioReagents®

Agaroses for DNA Electrophoresis

All Fisher BioReagents agaroses are DNase and RNase free to ensure optimal results for your nucleic acid application.

Three different grades of agarose are available which are functionally tested and pre qualified for the following specific applications:

- **Genetic Analysis Grade** – Agarose that yields biologically active DNA or RNA. Testing includes enzymatic performance measurements
- **Molecular Biology Grade** – Agarose that is suitable for analytical separation of DNA or RNA
- **PCR Grade** – Agarose that is suitable for the analytical separation of PCR amplicons (<1kb)

Agarose Selection Guide

Type of Agarose	Low EEO	Low Melting (>200bp)	Low Melting (<1kb)	Wide Separation Range	PCR Grade
Cat. No	10766834 (100g) 10366603 (500g)	10377033 (25g)	10583355 (100g)	10688973 (100g) 10776644 (500g)	10522775 (100g)
Recovery of DNA or RNA	•	•	•	•	•
Southern and Northern blots	•				
DNA/RNA separation 50bp to 1kb			•		•
DNA/RNA separation >1kb	•	•		•	
PCR fragment analysis	•	•	•	•	•
In-gel reactions ligation, transformations, PCR)			•		
Colony lifts	•				
Agarose Grade	Molecular Biology	Molecular Biology	Genetic Analysis	Genetic Analysis	PCR Grade



Buffers for DNA Electrophoresis Applications

Two buffers commonly used for DNA electrophoresis are Tris-acetate with EDTA and Tris-borate with EDTA. Because the pH of these buffers is neutral, the phosphate backbone of DNA has a net negative charge and migrates to the anode. TAE and TBE have different properties which makes one more suitable than the other for a specific purpose.

TAE: DNase, RNase and Protease free

Cat. No	Concentration	Quantity
10542785	1X	4L
10123293	1X	20L
10628403	10X	500mL
10041223	10X	1L
10775494	10X	4L
10775494	10X	20L
10255303	20X	1L**
10490074	25X	1L
10457583	50X	500mL
10490264	50X	1L
10542985	50X	4L
10326463	50X	20L

TBE: DNase and RNase free

Cat. No	Concentration	Quantity
10754914	1X	1L
10715684	1X	4L
10755104	1X	20L
11898562	5X	1L*
10727224	10X	1L
10031223	10X	4L
10563155	10X	20L
10448543	10X	1L**

*Pre-weighed powder in poly bottle. Dissolve in water

**Pre-weighed powder in foil pack. Dissolve in water

Buffer Components for DNA Electrophoresis

	Cat. No	Quantity
Tris Base	10103203	500g
	10376743	1kg
	10724344	5kg
	10667243	10kg
	10336793	25kg
Acetic Acid Glacial	10021123	500mL
	10522595	500g
Boric Acid	10011083	1kg
	10618973	500g
EDTA Disodium Salt	10618973	500g
	10522965	1kg



Fisher BioReagents®

Buffers for RNA Electrophoresis Applications

MOPS is a commonly used buffer system for RNA electrophoresis using formaldehyde or formamide-denatured RNA. It is important to use RNase free chemicals, water and containers when preparing the buffer solution. The typical formulation of 10X MOPS running buffer is 0.4M MOPS (pH 7.0), 0.1M sodium acetate and 0.01M EDTA.

MOPS: DNase, RNase and Protease free

Cat. No	Description	Quantity
10234673	Powder	100g
10234723	Powder	500g
10655025	10X buffer solution	500mL
11889191	10X buffer solution	1L
Water		
10295243	Nuclease free	50mL
10336503	Nuclease free	100mL
11448023	DNA grade	1L
10245203	RNA grade	1L

Gel Loading Agents

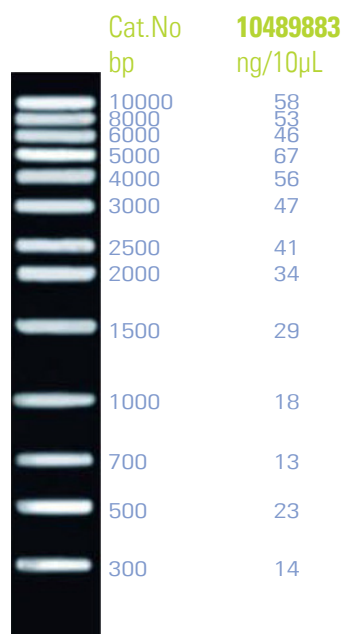
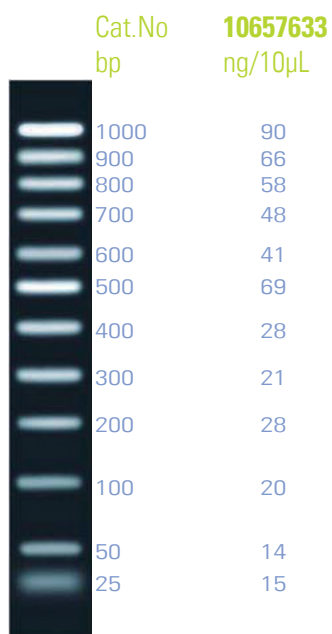
Cat. No	Concentration	Quantity
10205023	Agarose gel loading dye 6X	6mL
10205263	Glycerol gel-loading dye 5X DNase and RNase free	1mL
10400084	Glycerol gel-loading dye 5X DNase and RNase free	5mL
10679733	Bromophenol blue	25g
10532965	Xylene cyanol FF	10g

DNA Visualisation

Cat. No	Concentration	Quantity
10132863	Ethidium bromide solution 1%	10mL
10726074	Ethidium bromide	1g
10678973	Ethidium bromide	5g

General Bioreagents

Cat. No	Concentration	Quantity
10021123	Water, RNA grade, sterile, DNase RNase and protease free, DEPC treated, for RNA work	1L
10021123	Acetic acid glacial	500mL
10011083	Boric acid electrophoresis tested, DNase free	1kg
10021083	Glycerol, DNase, RNase and protease free	1L
10468343	Ficoll 400, m.w 400,000, DNase, RNase and protease free, molecular biology grade	100g



exACTGene™ and Routine DNA Ladders

Ready to use (pre mixed with loading dye), room temperature stable DNA ladders are available for all common electrophoresis applications.

exACTGene™ DNA ladders are ideal for qualitative analysis, quantitative estimation and size assessment

Cat. No	Application	Size Range	Number of Bands	Number of Loadings
10214973	PCR fragment analysis	25 to 650bp	14	100/10µL
10657633	PCR fragment analysis, small DNA digests	25 to 1,000bp	12	100/10µL
10224973	Quick check of PCR or enzyme digestion results	50 to 2,000bp	8	100/10µL
10061413	General purpose, small DNA fragments	100 to 1,000bp	10	100/10µL
10021463	Fast run times, small DNA fragments	100 to 2,000bp	11	100/10µL
10306943	Clone identification	100 to 2,686bp	14	100/10µL
10031463	Large size PCR or cloning	300 to 5,000bp	10	100/10µL
10122823	Small and large cloning application	100 to 5,000bp	16	100/10µL
10489883	General purpose, large digested DNA	300 to 10,000bp	13	100/10µL
10499883	General purpose, wide separation range	100 to 10,000bp	19	100/10µL
10699163	General purpose, extra large DNA fragments	300 to 24,000bp	15	100/10µL

Routine DNA ladders are designed for qualitative analysis and size assessment

Cat. No	Application	Size Range	Number of Bands	Number of Loadings
10284633	Small fragments, quick size assessment	50-2000bp	11	200/5µL
10450464	Quick size assessment of broad size range	50-10,000bp	16	200/5µL

Stock Solutions

50X TAE (Stock Solution)



To make 1L:

- Weigh out 242g Tris base (FW = 121) and dissolve in 750mL distilled water (Cat. No 10376743*)
- Add 57.1mL glacial acetic acid and 100mL 0.5M EDTA (pH 8.0) (Cat. No 10021123*) (Cat. No 10618973*)
- Make up to 1 liter with distilled water

Stock solution can be stored at room temperature. The pH of the buffer is not adjusted and should be approximately 8.5.

1X TAE (Working Solution)



Dilute stock solution by 50X in distilled water. Final concentrations are :

- 40mM Tris (pH 7.6)
- 20mM glacial acetic acid .
- 1mM EDTA

10X TBE (Stock Solution)



To make 1L:

- Weigh out 108g Tris base (FW = 121) and dissolve in 750mL distilled water (Cat. No 10376743*)
- Add 55g boric acid (FW = 61.8) and 40mL 0.5M EDTA (pH 8.0) (Cat. No 10011083*) (Cat. No 10618973*)
- Make up to 1 liter with distilled water

Stock solution can be stored at room temperature.

1X TBE (Working Solution)



Dilute stock solution by 10X in distilled water. Final concentrations are:

- 89mM Tris (pH 7.6)
- 89mM boric acid
- 2mM EDTA

6X DNA Loading Buffer



To make 100mL

- 60mL glycerol
- 6mL 1M Tris-HCl pH 8.0 (Cat. No. 10376743*)
- 1.2mL 0.5M EDTA, pH 8.0 (Cat. No. 10618973*)
- 32.8mL distilled water
- To the solution, add either 60mg of Bromophenol Blue (Cat. No 10679733*) or 60mg xylene cyanole FF (Cat. No 10532965*)

In a 1% agarose gel the tracking dyes are expected to run at approximately 300bp for bromophenol blue and 40,000bp for xylene cyanole.

Ethidium Bromide Solution



- Add 10mg of ethidium bromide (Cat. No 10678973*) to 1mL distilled water



CAUTION!

Ethidium bromide is a known mutagen. Always wear gloves when handling and wear a respiratory mask when weighing the powder. Wear UV safety goggles to protect skin and eyes when using any UV light source.

*refer to pages 14 to 18 for further details on these Fisher Bioreagents



Troubleshooting Guide

Most problems can be avoided by reading and following the instructions in this operating manual. Below we list some of those most commonly experienced along with suggestions for solving them. If, however, these should not resolve the issue, or if you have questions not covered below, please contact Fisher Scientific.

Problem	Suggestions
No bubbles appear at the electrodes when operating voltage is applied	<ul style="list-style-type: none">• Ensure that the d.c. power supply is properly connected
Melted agarose leaks when casting	<ul style="list-style-type: none">• When using casting gates, ensure that the sealing surfaces of the running tray and the gel casting gates are clean• Ensure that the ends of the running tray are flat and free of nicks
Sample well deformed	<ul style="list-style-type: none">• Allow the gel to set for a minimum of 30 minutes• Leave comb in position until gel returns to room temperature before removing• Remove the comb both slowly and at a slight angle to prevent gel from breaking• Avoid damaging the well with the pipettor when loading the sample; aim for the centre of the well and avoid damaging the bottom of the well with the pipettor tip
Samples leak underneath the gel upon loading	<ul style="list-style-type: none">• The bottom of the wells were torn when the comb was removed. To avoid tearing, carefully wiggle the comb to free the teeth from the gel
Samples do not run straight	<ul style="list-style-type: none">• Comb may be warped and should be replaced• Running tray may be warped and should be replaced• Reduce the voltage to reduce heat build up within gel• Choose a buffer with suitable ionic strength and buffering capacity
'Smiling' along one edge of the gel	<ul style="list-style-type: none">• Gel was not level when cast or run, use a gel levelling table to ensure that the apparatus is level before gel casting and electrophoresis

Problem

Suggestions

Bromophenol Blue dye turns yellow

- Check pH of buffer during electrophoresis (pH change)
- Ensure Tris base and not Tris-HCl was used
- Mix the buffer periodically during electrophoresis
- Connect a pump to circulate the buffer

Double banded pattern

- Ensure the comb is vertical during casting so that the well shape is not distorted
- Decrease the buffer level to 1mm above the top of the gel. This will reduce the temperature gradient through the gel
- Increase concentration of the sample and use a thin (2mm to 3mm) gel with a thin (1mm) comb

'Tailed' bands (excessive fluorescence appearing above the band)

- Reduce amount of nucleic acid in the sample

Poor band resolution

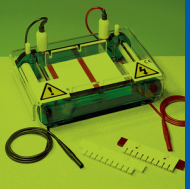
- Add Ficoll (Cat. No 10468343, refer to page 17), glycerol (Cat. No 10021083, refer to page 17), or sucrose to the sample loading buffer to ensure that the sample layers the bottom of the well. Ensure sample is completely dissolved
- Reduce voltage, sample concentration, or sample volume
- Ensure there is at least 1mm of gel below the bottom of the comb to prevent samples from leaking out the bottom of the well
- Reduce salt concentration of the sample. High salt concentrations can cause 'pinched' lanes, smeared lanes, arched dye front and slow migration
- Check enzyme activity; may require longer digestion or different restriction buffer
- Prepare fresh sample if nuclease contamination is suspected
- Choose agarose with low endosmosis value

Gel melts or softens near sample wells

- Caused by a combination of pH drift and high temperature. Circulate or remix buffer periodically or reduce the voltage

References

- Sambrook, Fritsch, and Maniatis, Molecular Cloning A Laboratory Manual, Second Edition
- Cold Spring Harbor Laboratory Press, 1989
- Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley-Interscience, 1989



Warranty

- Fisher Scientific SUB-GEL Horizontal Electrophoresis units have a warranty against manufacturing and material faults of twelve months from date of customer receipt
- If any defects occur during this warranty period, your supplier will repair or replace the defective parts free of charge
- This warranty does not cover defects occurring by accident or misuse, or defects caused by improper operation
- Units where repair or modification has been performed by anyone other than your supplier or an appointed distributor or representative are no longer under warranty from the time the unit was modified
- Units which have accessories or repaired parts not supplied by your supplier or its associated distributors have invalidated warranty
- Your supplier cannot repair or replace free of charge units where improper solutions or chemicals have been used. For a list of these please see the Care and Maintenance subsection

If a problem does occur then please contact your nearest Fisher Scientific supplier



Warning

DO NOT attempt to remove the outer casing or make repairs to our electrical range of products, should any unit fail. Contact Fisher Scientific immediately if the need for repair or servicing should arise.

NOTES

A series of horizontal dotted lines for taking notes.

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