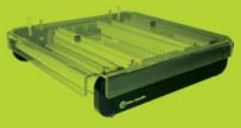


Fisherbrand®
QUALITY. RELIABILITY. VALUE.

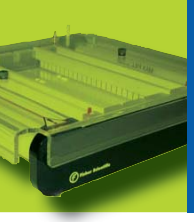
Instruction Manual

Horizontal Electrophoresis Units, wide format

11553352
wide format, Mini-Plus

11563382
wide format, Midi-Plus

Horizontal Electrophoresis Units



Fisherbrand®
QUALITY. RELIABILITY. VALUE.

INSTRUCTION MANUAL

Horizontal Electrophoresis Units, wide format

TABLE OF CONTENT

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

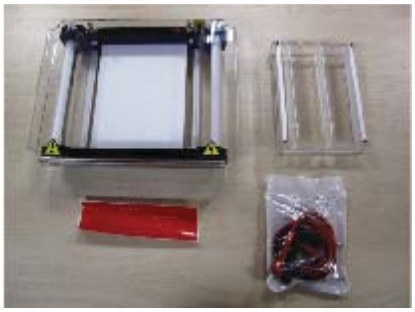
Packing List

Cat. No	Plastic Gel Casting Gates	Comb Thickness Teeth No. & Qty	Buffer Recirculation Ports	Gel Casting Tray	Checked
11553352 (FHU10WMK2)	**11573352** (FHU10W2CG)	**11573362** (FHU10W2C120) x 2 1mm, 20 sample	-	**11563352** (FHU10W2UT)	
11563382 (FHU13WMK2)	**11503392** (FHU13W2CG)	**11553392** (FHU13W2C125MC) x 2 1mm, 25 sample	**11593382** (FHUBRP)	**11583382** (FHU13W2UT14)	

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.

Horizontal gel unit, wide format, Mini-Plus (11553352)



Horizontal gel unit, wide format, Midi-Plus (11563382)

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
- Wide Format Midi-Plus unit supplied with one casting tray and with casting gates and integral silicone seals
- 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 Horizontal 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 (H_2O_2) for 10 minutes
- Rinse with 0.1% DEPC (diethyl pyrocarbonate) treated distilled water (Cat. No 10245203, refer to page 15)

Caution

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

Using the Horizontal Gel Electrophoresis Units

Setting Up the Horizontal Gel Tanks

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 Pouring

- Make sure that the silicone rubber gasket is in position, i.e. pushed evenly into the groove around the edges of the gel casting gates
- Place the casting gates into the slots provided in the gel casting tray with the end silicone rubber gaskets facing outwards (Fig 1)
- Place the gel unit on a level surface, or a gel levelling table
- Position the required combs into the slots provided within the gel casting tray (Fig 2)
- Prepare 75 and 160mL volumes of the desired % agarose for units 11553352 and 11563382 respectively. This will give a gel depth of 5mm. For a 10mm deep gel prepare double the volume (refer to page 14, Agarose Selection Guide)
- Pour in the agarose smoothly to prevent bubbles. If bubbles do occur, these can be smoothed to the side of the gel and dispersed using a clean gloved hand.
  (IMPORTANT: ensure that the agarose has cooled to between 50 and 60°C before pouring to prevent apparatus distortion)
- Allow the agarose to set, ensuring that the gel remains undisturbed (Fig 3)
- Carefully remove the gel casting gates and comb(s)
- Place the gel casting tray in the running position such that the wells are nearest to the black electrode (cathode)
- Fill the tank with running buffer so that the buffer just submerges the gel (refer to page 14 for details on Buffers for Electrophoresis Applications).

Fig.1

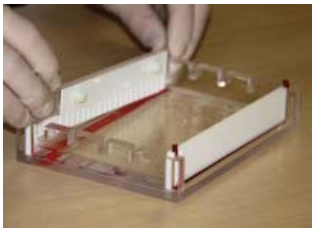


Fig.2

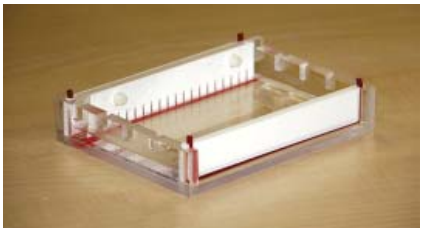


Fig.3

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 16 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. See Operating Conditions table below for recommended voltage and current settings. 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
- Long runs may require buffer recirculation, to prevent overheating and or buffer depletion. Recirculation ports are provided on the 11563382 unit. IMPORTANT: When recirculating buffer, remember that the buffer flowing through the tubing is live. Take all necessary precautions. Warn other workers in the vicinity of the potential hazard. Seek the advice of your Safety Officer

Operating Conditions

Cat. No	Normal Operating Voltage (V)	Normal Operating Current (mA)	Gel Vol. for a 5mm Gel (mL)	Approx. Buffer Volume (mL)	Electrode Separation (mm)
11553352	150	100	75	400	160
11563382	150	100	160	1,000	200

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, a 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 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 14 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 18 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 (0.5 µg/mL H_2O) 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 $MgCl_2$, 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 units 11553352 and 11563382.

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.

11553352 - Horizontal Gel Unit, Wide Format, Mini-Plus

Cat. No	Thickness (mm)	No. of Samples	Tooth Width (mm)	Tooth Spacing (mm)	Max. Sample Vol. Per well for a 5mm Deep gel, µL
11523362	1	4	33	2	41.9
11533362*	1	8	15.5	2	66.65
11543362	1	10	12	2	51.6
11553362	1	12	9.4	2.3	40.42
11563362*	1	16	6.8	2	29.24
11573362	1	20	5	2	21.5
11503372	1.5	4	33	2	212.85
11513372*	1.5	8	15.5	2	99.975
11523372	1.5	10	12	2	77.4
11533372	1.5	12	9.4	2.3	60.63
11543372*	1.5	16	6.8	2	43.86
11553372	1.5	20	5	2	32.25
11583372	2	4	33	2	283.8
11593372*	2	8	15.5	2	133.3
11503382	2	10	12	2	103.2
11513382	2	12	9.4	2.3	80.84
11523382*	2	16	6.8	2	58.48
11533382	2	20	5	2	43

* denotes that these combs are multichannel pipettor compatible

11563382 - Horizontal Gel Unit, Wide Format, Midi-Plus

Cat. No	Thickness (mm)	No. of Samples	Tooth Width (mm)	Tooth Spacing (mm)	Max. Sample Vol. Per Well for a 5mm Deep Gel, µL
11523392*	1	12	16.7	2.2	71.81
11533392	1	16	12.1	2.0	52.03
11543392	1	20	9.3	2.0	39.99
11553392*	1	25	7.0	2.0	30.1
11563392	1	28	6.0	2.1	25.8
11573392	1	40	3.9	1.8	16.77
11583392*	1	50	3.5	1.0	15.05
11593392*	1.5	12	16.7	2.2	107.715
11503402	1.5	16	12.1	2.0	78.045
11513402	1.5	20	9.3	2.0	59.985
11523402*	1.5	25	7.0	2.0	45.15
11533402	1.5	28	6.0	2.1	38.7
11543402	1.5	40	3.9	1.8	25.155
11553402	1.5	50	3.5	1.0	22.575
11563402*	2	12	16.7	2.2	143.62
11573402	2	16	12.1	2.0	104.06
11583402	2	20	9.3	2.0	79.98
11593402	2	25	7.0	2.0	60.2
11503412	2	28	6.0	2.1	51.6
11513412	2	40	3.9	1.8	33.54
11523412*	2	50	3.5	1.0	30.1

* denotes that these combs are multichannel pipettor compatible

Fisher BioReagents™

Your source for high purity products for nucleic acid electrophoresis.

Packaging for Safety, Convenience and Product Quality

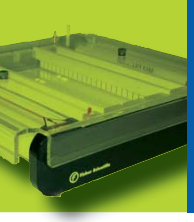
Fisher Bioreagents come in a wide variety of innovative packaging designed for safety, environmental protection, convenient handling and storage, and preservation of product integrity. The primary container is included in the product description of most chemicals in this catalogue.

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.



Agaroses for DNA Electrophoresis

Fisher BioReagents®

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
10490074	25X	1L
10457583	50X	500mL
10490264	50X	1L
10542985	50X	4L
10326463	50X	20L
10255303	20L	1L**

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

Fisher BioReagents®

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

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

Fisher BioReagents®

Gel Loading Agents

Cat. No	Concentration	Quantity
10205023	Agarose gel loading dye 6X	6 mL
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%	10 mL
10726074	Ethidium bromide	1 g
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

Cat.No	**10657633**
bp	ng/10µL
1000	90
900	66
800	58
700	48
600	41
500	69
400	28
300	21
200	28
100	20
50	14
25	15

Cat.No	**10489883**
bp	ng/10µL
10000	58
8000	53
6000	46
5000	67
4000	56
3000	47
2500	41
2000	34
1500	29
1000	18
700	13
500	23
300	14

Fisher BioReagents®

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	200/5uL
10450464	Quick size assessment of broad size range	50-10,000bp	16	200/5uL

Stock Solutions

50X TAE (Stock Solution)

Fisher BioReagents®

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)

Fisher BioReagents®

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)

Fisher BioReagents®

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)

Fisher BioReagents®

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

- 89mM tris (pH 7.6) (Cat. No 10376743*)
- 89mM boric acid (Cat. No 10011083*)
- 2mM EDTA (Cat. No 10618973*)

6X DNA Loading Buffer

Fisher BioReagents®

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

Fisher BioReagents®

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

Ethidium bromide is a known mutagen. Always wear gloves when handling.

Wear UV safety goggles and protect skin when using any UV light source

*refer to pages 13 to 17 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	• Ensure that the d.c. power supply is properly connected
Melted agarose leaks when casting	• 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	• 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	• 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	• Comb may be warped should be replaced • Running tray may be warped 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	• 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
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

Problem	Suggestions
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 16), glycerol (Cat. No 10021083, refer to page 16), 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

- Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
- Rickwood, D. and Hames, B. D. (eds.) (1982) Gel Electrophoresis of Nucleic Acids: A Practical Approach, IRL Press, Oxford, England
- Longo, M. C. and Hartley, J. L. (1986) Focus 8:3, 3
- Ausubel, et al., (eds). (1993) Current Protocols in Molecular Biology. Greene Publishing and Wiley Interscience, New York

Warranty

- Fisher Scientific's 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 units free of charge 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

NOTES

Fisherbrand®
QUALITY. RELIABILITY. VALUE.

Fisherbrand Fits Everywhere

Helping you to produce your best work each and every day

For our FULL RANGE of
FISHERBRAND PRODUCTS visit
www.eu.fishersci.com/fisherbrand

Austria: +43(0)800-20 88 40 **Belgium:** +32 (0)56 260 260 **Denmark:** +45 70 27 99 20
Germany: +49 2304 932-5 **Ireland:** +353 (0)1 885 5854 **Italy:** +39 02 950 59 478
Finland: +358 (0)9 8027 6280 **France:** +33 (0)3 88 67 14 14 **Netherlands:** +31 (0)20 487 70 00
Norway: +47 22 95 59 59 **Portugal:** +351 21 425 33 50 **Spain:** +34 902 239 303
Sweden: +41 31 352 32 00 **Switzerland:** +41 (0)56 618 41 11 **UK:** +44 (0)1509 555 500

Fisher Scientific
A Thermo Fisher Scientific Brand

SE_EN_ 2013_161_FB_favourites