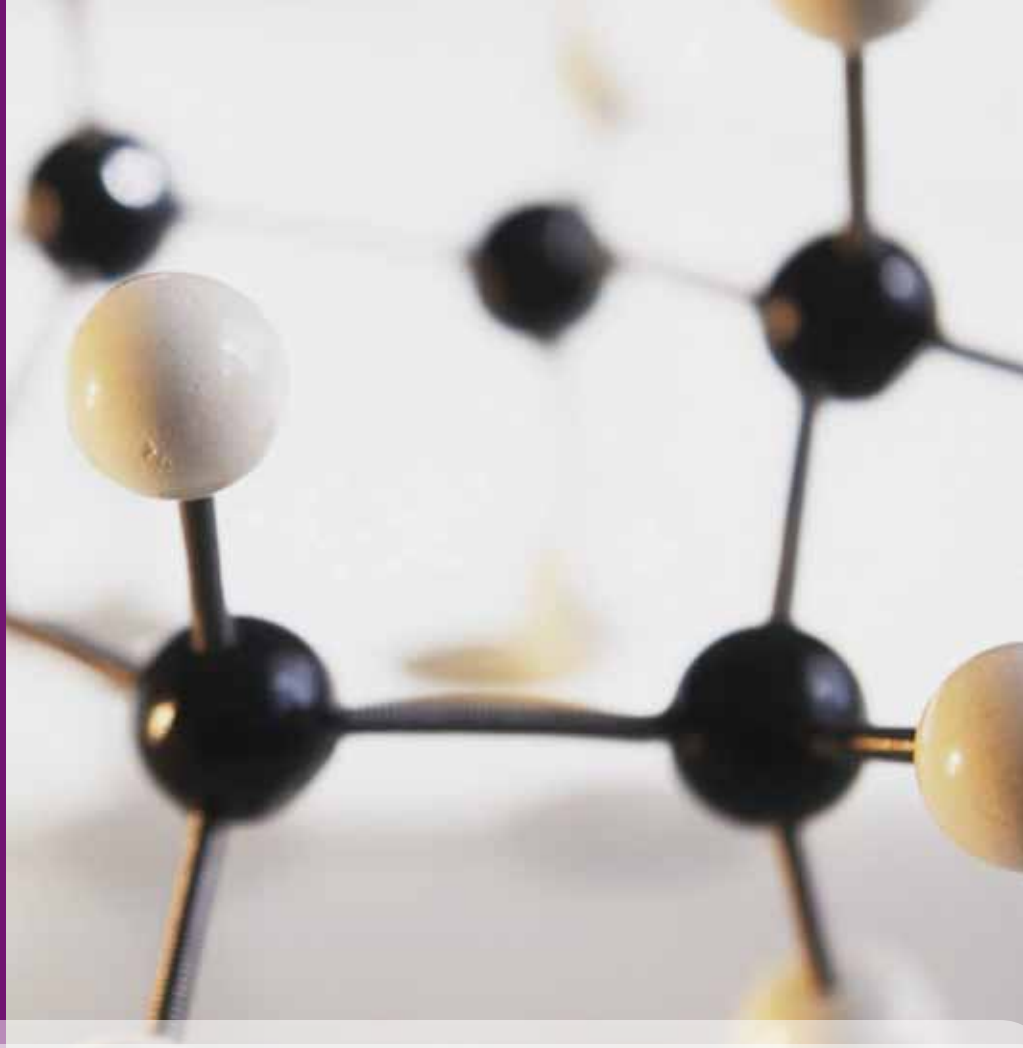
Fisher Scientific

AGAROSE

BUFFERS

LADDERS

EQUIPMENT



Nucleic Acid Electrophoresis

APPLICATION GUIDE

Fisher Chemical

Fisher BioReagents®

Thermo SCIENTIFIC

REAGENTS: AGAROSE

Thermo Scientific and Fisher Scientific products deliver an end-to-end solution that can meet your most demanding electrophoresis requirements.

You can depend on our expertise in electrophoresis instruments along with ultrapure reagents that are pre-qualified for your applications. This guide is designed to help you select the right products from our best-in-class array of laboratory equipment and bioreagents.

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

Fisher BioReagents offers three different grades of agarose that are functionally tested and pre-qualified for specific applications.

Agarose grades used in electrophoresis of nucleic acids:

Genetic Analysis Grade—agarose that yields biologically active DNA or RNA. Testing includes enzymatic performance measurements.

Molecular Biology Grade—suitable for analytical separation of DNA or RNA.

PCR Grade—the original agarose for analytical separation of PCR amplicons (<1kb).

Agarose is a linear polysaccharide composed of alternating residues of D- and L-galactose joined by glycosidic linkages. Agarose forms gels that are both porous and resilient.

These gel properties provide a sieving matrix that allows the electrophoretic separation of charged macromolecules such as DNA or RNA according to size. Compared to polyacrylamide gel, agarose has a lower resolution but wider range of separation.

Lower grades of agarose can be contaminated with other polysaccharides, salts, and proteins. Such impurities can alter the gelling/melting temperature of agarose solutions or affect the ability to use the recovered nucleic acid sample in a post-electrophoresis application.

3-STEP Selection Process

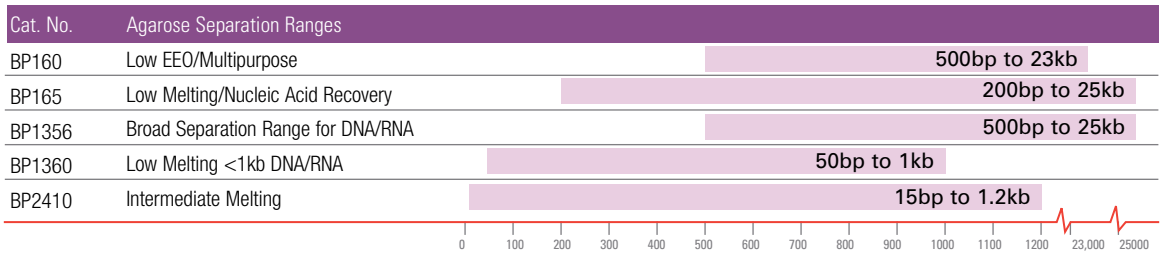
Separation of Nucleic Acids by AGAROSE GEL ELECTROPHORESIS

1. Choose Your Reagents
   - Agarose
   - Buffer
   - Ladders
2. Choose Your Equipment
   - Power Supply
   - Gel Box
3. Downstream Application Essentials
   - Gel Staining
   - Hybridization
   - DNA Gel Extraction

Two Factors for Selecting an Agarose

1. The size of DNA or RNA fragments to be analyzed (see graph below).

Cat. No.	Agarose Separation Ranges
BP160	Low EEO/Multipurpose
BP165	Low Melting/Nucleic Acid Recovery
BP1356	Broad Separation Range for DNA/RNA
BP1360	Low Melting <1kb DNA/RNA
BP2410	Intermediate Melting



2. The type of downstream application that will follow electrophoretic separation (e.g., cloning procedures directly from remelted agarose or in-gel reaction).

Agarose Selection Guide

Type of Agarose	Low EEO	Low Melting >200bp	Low Melting <1000bp	Wide Separation Range	PCR Grade
Cat. No.	**BP160**	**BP165**	**BP1360**	**BP1356**	**BP2410**
Recovery of DNA and RNA	x	x	x	x	x
Southern and Northern Blots	x				
DNA/RNA separation 50bp to 1kb			x		x
DNA/RNA separation >1kb	x	x		x	
PCR fragment analysis	x	x	x	x	x
In-gel reactions (ligation, transformations, PCR)			x		
Colony lifts	x				
Available pack sizes	100g and 500g	25g	100g	100g and 500g	100g
Agarose grade	Molecular biology	Molecular biology	Genetic analysis	Genetic analysis	PCR

Two buffers commonly used for DNA agarose electrophoresis are Tris-acetate with EDTA (TAE; 40mM Tris-acetate, 1mM EDTA) and Tris-borate with EDTA (TBE, 89mM Tris-borate, 2mM EDTA). Because the pH of these buffers is neutral, the phosphate backbone of DNA has a net negative charge and migrates toward the anode. TAE and TBE have different properties which makes one more suitable than the other for a specific purpose.

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 a 10X MOPS running buffer is 0.4M MOPS (pH 7.0), 0.1M sodium acetate, and 0.01M EDTA.

The denaturing system chosen depends on the purpose of the RNA experiment and the size of the RNA fragment being separated. Formaldehyde denaturation is suitable if RNA samples are to be recovered. Formamide denaturation is suitable if the RNA needs to retain its biological activity.

Buffer	Suggested Uses	Properties
TAE	DNA recovery. Electrophoresis of large DNA (>12kb).	Low buffering capacity. Recirculation may be necessary for extended run times (>6 hr.)
TBE	Electrophoresis of small DNA (<1kb). Increased resolution of small DNA (<1kb).	Decreased DNA mobility. High buffering capacity – no recirculation required for extended run times.
MOPS	Electrophoresis of formaldehyde denatured RNA.	Buffer is low in ionic strength. Recirculation of buffer may be necessary.

Suggested Agarose Concentrations

The optimal gel concentration depends on the size of the DNA fragments to be resolved.

Cat. No.	Main Application	DNA Size Range in Base Pairs	Final Agarose Concentration % (W/V) 1x TAE buffer	Final Agarose Concentration % (W/V) 1x TBE buffer
BP1360	Low melting temperature agarose.	500-1,000	2.5	2.0
	Certified recovery of small nucleic	150-700	3.0	2.5
	acid fragments.	100-450	3.5	3.0
	Outstanding resolution.	70-300	4.0	3.5
		10-100	4.5	4.0
		8-50	5.0	4.5
BP165	Low melting temperature agarose.	500-25,000	0.75	0.70
	Broad separation range.	300-20,000	1.00	0.85
	Ideal for DNA and RNA recovery	200-12,000	1.25	1.00
	after electrophoretic separation.	150-6,000	1.50	1.25
		100-3,000	1.75	1.50
		50-2,000	2.00	1.75
BP1356	Suitable for routine nucleic acid	1,000-23,000	0.60	0.50
BP160	electrophoresis applications with	800-10,000	0.80	0.70
	broad separation range.	400-8,000	1.00	0.85
		300-7,000	1.20	1.00
		200-4,000	1.50	1.25
		100-3,000	2.00	1.75

NEW!

Ethanol, Molecular Biology Grade, is an ultrapure molecular biology grade ethanol that is DNase-, RNase- and Protease-free. It is used for the purification and precipitation of biomolecules such as nucleic acids and proteins.

Cat. No.	Size
BP2818-100	100mL
BP2818-500	500mL
BP2818-4	4L

NEW!

Water, Molecular Biology Grade, is ideal for many fundamental procedures such as PCR, electrophoresis, DNA sequencing and buffers for enzymatic analyses.

Cat. No.	Size
BP2819-100	100mL
BP2819-1	1L
BP2819-4	4L
BP2819-10	10L
BP2819-20	20L

Buffers for Nucleic Acid Applications

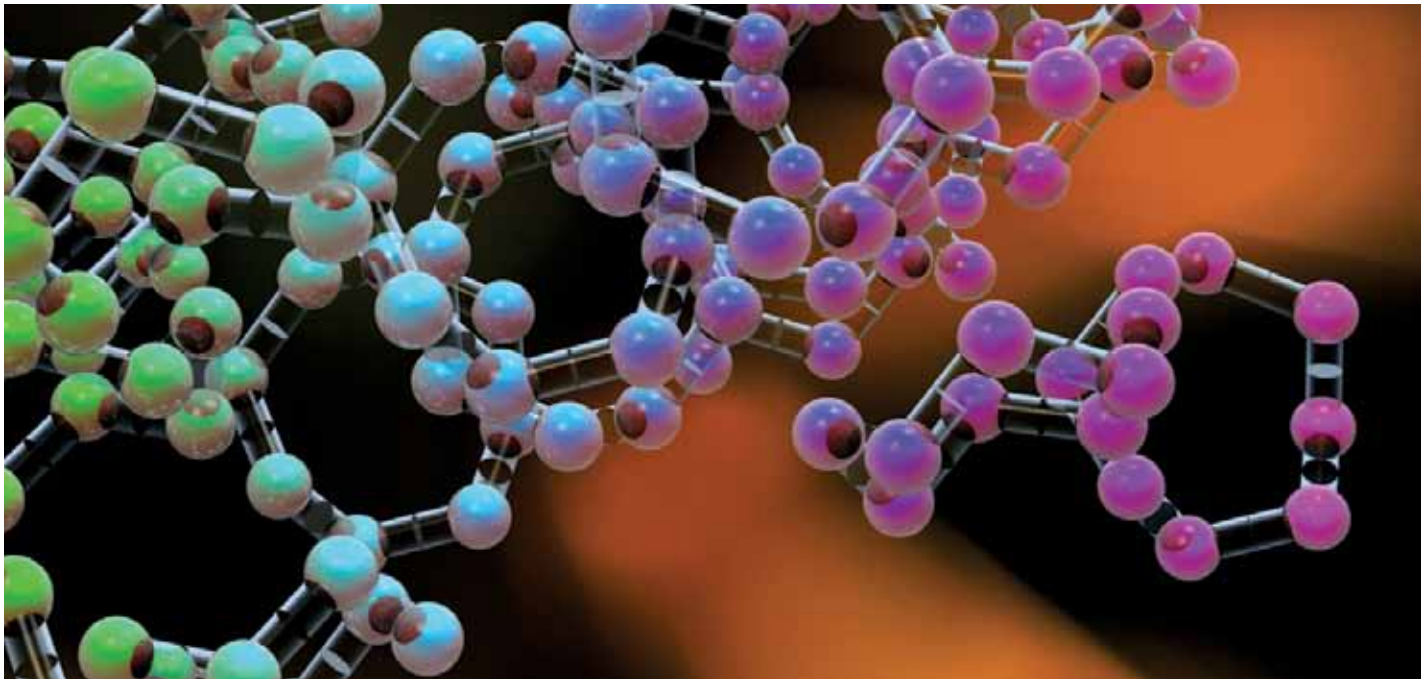
Cat. No.	Concentration	Size
TBE		
BP2430-1	1X	1L
BP2430-4	1X	4L
BP2430-20	1X	20L
BP1396-86	5X	1L*
BP1333-1	10X	1L
BP1333-4	10X	4L
BP1333-20	10X	20L
BP1334-1	10X	1L**
TAE		
BP2434-4	1X	4L
BP2434-20	1X	20L
BP1335-500	10X	500mL
BP1335-1	10X	1L
BP1335-4	10X	4L
BP1335-20	10X	20L
BP1330-1	25X	1L
BP1332-500	50X	500mL
BP1332-1	50X	1L
BP1332-4	50X	4L
BP1332-20	50X	20L
BP1331-1	25X	1L**

Cat. No.	Description	Size
MOPS		
BP308-100	Powder	100g
BP308-500	Powder	500g
BP2900-500	10x Buffer Solution	500mL
BP2900-1	10x Buffer Solution	1L
WATER		
BP2484-50	Nuclease-Free	50mL
BP2484-100	Nuclease-Free	100mL
BP2470-1	DNA-Grade	1L
BP561-1	RNA-Grade	1L
FORMALDEHYDE		
BP531-25	37% by weight	25mL
BP531-500	37% by weight	500mL

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

In addition to premixed buffers, Fisher BioReagents also offers individual buffer components for you to create your own buffer recipe

Buffer Component	Size	Cat. No.
Tris Base	500g	**BP152-500**
	1kg	**BP152-1**
	5kg	**BP152-5**
	10kg	**BP152-10**
	25kg	**BP152-25**
Glacial Acetic Acid	500mL	**BP2401-2500**, **BP2401S-500** (Safe-Cote®)
	2.5L	**BP2401-212**, **BP2401SI-212** (Safe-Cote)
Boric Acid	500g	**BP168-500**
	1kg	**BP168-1**
EDTA Disodium Salt	500g	**BP120-500**
	1kg	**BP120-1**



To achieve the most accurate qualitative and quantitative analysis via agarose gel electrophoresis, the appropriate DNA or RNA standard is required.

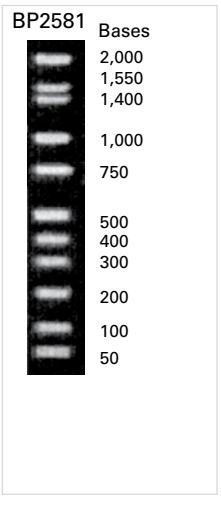
Fisher BioReagents provides a wide range of standards, including routine DNA ladders for quick size and quality assessment as well as *ex*ACTG*ene*® DNA ladders that allow for quantitative analysis.

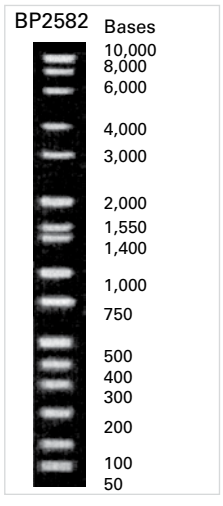
RiboLadders™ RNA Standards

These standards can be used to assess single-stranded RNA molecules on both native and denaturing agarose gels. These unique RNA standards are lyophilized to reduce thawing-related degradation, to prolong shelf life and to ensure consistent performance.

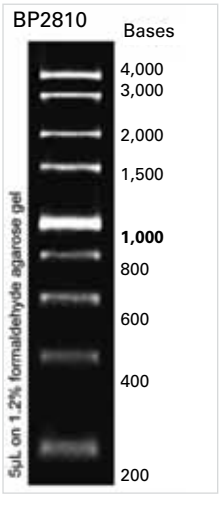
Cat. No.	Application	Size Range	Number of Bands	Number of Loadings
	Sizing unknown RNA fragments			
BP2810-50	Small RNA fragments	0.1 – 1kb	8	50
BP2811-50	Large RNA fragments	0.2 – 4kb	9	50

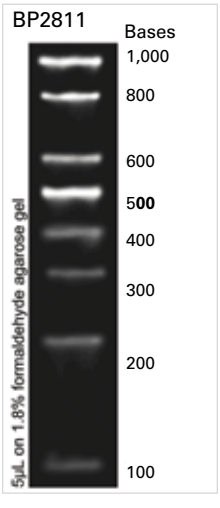
Routine DNA Ladders





RiboLadders RNA Standards





REAGENTS: LADDERS

*ex*ACTG*ene*® and Routine DNA Ladders

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

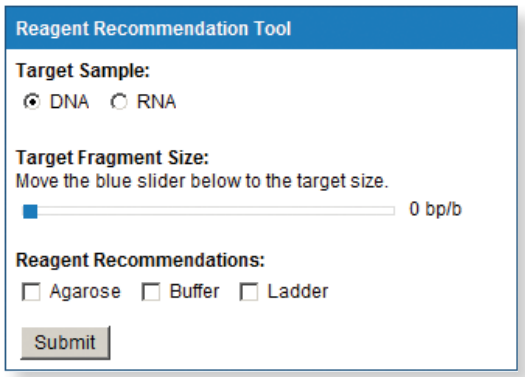
NEW!

On Chemical Mini-site

Reagent Recommendation Tool for Nucleic Acid Electrophoresis

One click to see the ideal reagents for your specific application

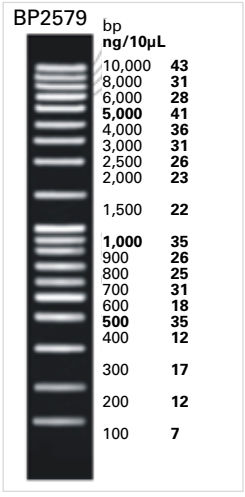
www.fishersci.com/ NucleicAcidElectrophoresis

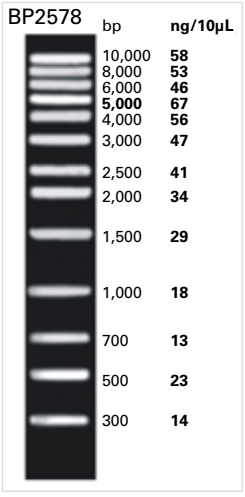


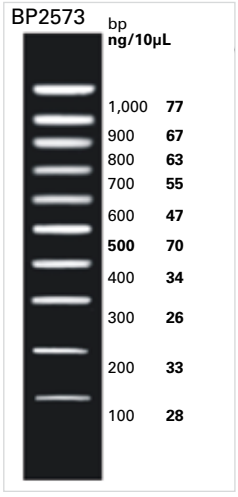
Cat. No.	Application	Size Range	Number of Bands	Number of Loadings
	***ex*ACTG*ene* DNA ladders are ideal for qualitative analysis, quantitative estimation and size assessment**			
BP2570-100	PCR fragment analysis	25-650bp	14	100/10uL
BP2571-100	PCR fragment analysis, small DNA digests	25-1000bp	12	100/10uL
BP2572-100	Quick check of PCR or enzyme digestion results	50-2000bp	8	100/10uL
BP2573-100	General purpose, small DNA fragments	100-1000bp	10	100/10uL
BP2574-100	Fast run times, small DNA fragments	100-2000bp	11	100/10uL
BP2575-100	Clone identification	100-2686bp	14	100/10uL
BP2576-100	Large size PCR or cloning	300-5000bp	10	100/10uL
BP2577-100	Small and large cloning application	100-5000bp	16	100/10uL
BP2578-100	General purpose, large digested DNA	300-10,000bp	13	100/10uL
BP2579-100	General purpose, wide size range	100-10,000bp	19	100/10uL
BP2580-100	General purpose, extra-large fragments	300-24,000bp	15	100/10uL
	Routine DNA ladders are designed for qualitative analysis and size assessment			
BP2581-200	Small fragments, quick size assessment	50-2000bp	11	200/5uL
BP2582-200	Quick size assessment of broad size range	50-10,000bp	16	200/5uL

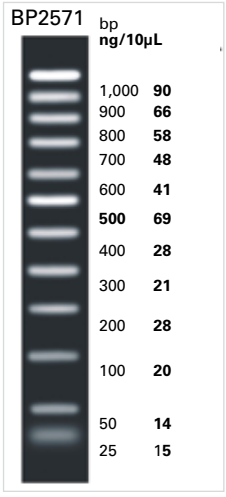
For Lambda DNA digests or other DNA markers and ladders not containing loading dye, please visit **www.fishersci.com** and type BP2553-100 in the search box.

*ex*ACTG*ene* DNA Ladders









EQUIPMENT: GEL BOXES

Submarine Electrophoresis Gel Boxes

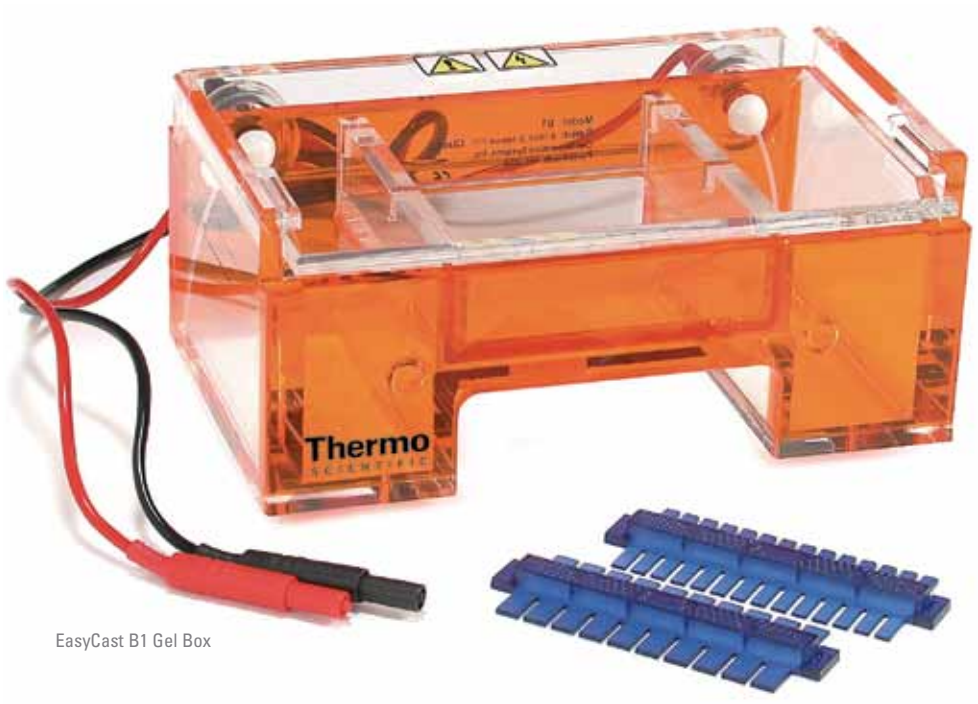
Thermo Scientific submarine electrophoresis gel boxes are available in three sizes and sample quantities. This gel size flexibility is important in order to provide sufficient separation of closely spaced DNA fragments as well as small and large nucleic acid fragments on the same gel.

Thermo Scientific EasyCast® Systems contain:

- Compact footprint
- Thermo Scientific EasyCast gasket UVT gel tray
- Thermo Scientific SuperSafe® lid with attached power supply leads
- Optional cooling system

Thermo Scientific Millipede® Systems contain:

- Buffer chamber with 3-point leveling base
- Thermo Scientific SuperSafe lid with attached power supply leads
- UV transparent gel tray with gasket end gates
- Leveling bubble



EasyCast B1 Gel Box

Cat. No.	Model	Cooling Option	Max. Number of Samples	Gel Size (W x L) in cm	Running Buffer Volume in mL
09-528-178	Thermo Scientific EasyCast B1	No	34	9 x 11	600
OWB1BP	Thermo Scientific EasyCast B1	Yes	34	9 x 11	600
09-528-110B	Thermo Scientific EasyCast B2	No	108 with rapid load tray	12 x 14	800
09-528-118	Thermo Scientific EasyCast B2	Yes	108 with rapid load tray	12 x 14	800
09-528-124	Thermo Scientific EasyCast B3	Yes	300 with rapid load tray	12 x 14	1000
OWA6	Thermo Scientific Millipede A6	No	500	23 x 25	3000

Voltage Table

The table (below) provides recommended voltages and buffers according to DNA size and application. The distance used to determine the voltage gradients is the distance between electrodes, not the gel length. If the voltage is too high, band streaking may occur for large DNA sizes (>12kb). When the voltage is too low, the mobility of small (<1kb) DNA is reduced, and band broadening will occur due to dispersion and diffusion.

Gel Size	Voltage	Recovery Buffer	Analytical Buffer
<1kb	5V/cm	TAE	TBE
<1kb to >12kb	4-10V/cm	TAE	TBE
>12kb	1-2V/cm	TAE	TAE

Fisher Scientific

	Fisher Scientific US	Fisher HealthCare US	Fisher Scientific Global Export, Latin America	Fisher Scientific Canada
For customer service	1-800-766-7000	1-800-640-0640	1-770-871-4725	1-800-234-7437
To fax an order	1-800-926-1166	1-800-290-0290	1-770-871-4726	1-800-463-2996
To order online	www.fishersci.com	www.fisherhealthcare.com	www.fishersci.com	www.fishersci.ca

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