Product Specifications

Electrophoresis Reagents, Buffers, Agarose, Polymerase Chain Reaction
Custom Primers and Probes
Hybridization and Detection Reagents

Agarose HiRes Molecular Biology Grade

Store at Room Temperature

	Catalog Number	Description	Size
❑	40-3015-10	Agarose HiRes Ultra Pure Molecular Biology Grade	100 gms
❑	40-3015-50	Agarose HiRes Ultra Pure Molecular Biology Grade	500 gms
❑	40-3015-01	Agarose HiRes Ultra Pure Molecular Biology Grade	1 KG

Product Description & Application

Agarose HiRes is certified Ultra Pure molecular biology grade DNase and RNase-free agarose powder. It is specifically recommended for resolution of short fragments ranging in size between 20 bp and 800 bp and is an excellent substitute for polyacrylamide electrophoresis for resolution of short DNA fragments. HiRes Agarose is commonly used for electrophoretic resolution of fragments obtained from amplification of short tandem repeats (STR's), di, tri and tetra-nucleotide repeats, and other polymorphic loci.

Specifications

Appearance	White homegeous powder
Gel strength of 1.5 % (w/v) gel	>1680g / cm_2
Gel strength of 3 % (w/v) gel	>3290g / cm_2
Gelling temperature	33-34°C
Melting temperature	74°C
EEO:	0.1-0.2
Moisture:	<4%
DNase and RNase	None detected

High Resolution Gel Electrophoresis of DNA

Gene Link HiRes agarose is an intermediate melting temperature agarose (~74°C) that provides one of the finest resolutions for DNA fragments from STR, tri and tetra-nucleotide repeat amplification and other length based polymorphisms.

Using a 2 – 4% gel (made in either TAE or TBE) it is possible to resolve fragments that are anywhere from 20 – 800 bp in length. A 4% HiRes agarose gel can differentiate a 99bp fragment from a 110 bp fragment running the gels at 45 mAmps at room temperature. A 3% HiRes gel can also be used for routine short fragment PCR electrophoresis and recovery of DNA fragments from the gel.

Resolution & Percentage HiRes Agarose Gel Preparation		
Size Range	**Final Agarose Concentration (%)**	
(Base Pairs)	1X TAE Buffer	1X TBE Buffer
150-800	2.0	1.8
100-600	3.0	2.0
50-250	4.0	3.0
20-130	5.0	4.0
<80	--	5.0

Approximate Dye Mobility in HiRes Agarose				
1X TAE Buffer		**%**	**1X TBE Buffer**	
XC*	BPB**	Agarose	XC	BPB
480 bp	70 bp	2.0	310 bp	40 bp
200 bp	40 bp	3.0	140 bp	35 bp
120 bp	35 bp	4.0	85 bp	30 bp
85 bp	30 bp	5.0	60 bp	15 bp
*XC, xylene cyanol; **BPB, bromophenol blue				

Preparation of HiRes Agarose Gel

Follow routine laboratory safety procedures for preparation of agarose gel using either microwave or boiling water bath method. The following additional steps should be followed specifically for the preparation of HiRes agarose gels.

1. Soak the weighed out agarose powder in the buffer solution for 15 minutes to gently swell and form a homogenously dispersed mixture. Gently stir by hand to uniformly spread the agarose powder. Clumps should be totally broken.
2. Heat in microwave in installments and gently swirl to reduce foaming.
3. After complete dissolution transfer flask on bench top to cool. Gently swirl intermittently to reduce cool spots.
4. The gel should be cooled to ~65°C prior to casting. Pour gel and let stand at room temperature for nearly 15 minutes for gel to set.
5. **IMPORTANT: It is essential to place gel at 4° C for 20 minutes to harden and cure. This process yields optimal resolution and gel handling characteristics.**

Routine Gel Electrophoresis of DNA

Gel electrophoresis of PCR products is the standard method for analyzing reaction quality and yield. PCR products can range up to 10 kb in length, but the majority of amplifications are at 1 kb and below. Agarose electrophoresis is the classical method to analyze amplification products from 150 bp to greater than 10 kb. Polyacrylamide gel electrophoresis should be used for resolution of short fragments in the range of 100 bp to 500 bp when discrimination of as small as a 10 bp difference is required.
Polyacrylamide gels for PCR products can be formulated with the amount of cross-linker chosen to give pore sizes optimal for the size of DNA fragment desired. Gels are most often stained in ethidium bromide, even though the fluorescence of this stain is quenched by polyacrylamide, which decreases sensitivity 2-5 fold. This decrease in sensitivity generally does not present a problem, because most PCR reactions yield product levels in the microgram range and ethidium will detect as little as 1/10 of this amount. Polyacrylamide gels can be stained by silver staining for more sensitive detection.

Agarose Gel Electrophoresis of DNA

Agarose gels are typically run at 20 to 150V. The upper voltage limit is the amount of heat produced. At room temperature about 5 Watts is correct for a minigel (Volts x Amps = Watts). At low voltages migration is linearly proportional to voltage, but long DNA molecules migrate relatively faster in stronger fields. Migration is inversely proportional to the log of the fragment length; a log function also governs migration rate and gel concentration (0.5 to 2% for most purposes). Furthermore, supercoiled / circular DNA molecules migrate at different rates from linear molecules; single-stranded DNA and RNA migrate at similar rates, but usually faster than double-stranded DNA of the same length. Salt in the samples increases conductivity and, hence, migration rate.
The buffers used for most neutral agarose gels (the gel itself and the solution in which it lies) is 1 x TAE or 1 x TBE. Agarose powder is added to the buffer at room temperature, heated in a microwave and boiled slowly until the powder has dissolved. Cast the gel on a horizontal surface once the agarose has been cooled to ca. 60° C (just cool enough to hold) and add 0.1 µg of ethidium bromide solution for each ml of gel volume. At times, during removal of the comb, it is possible to tear the bottom of the sample wells gels, which results in sample leakage upon loading. This can be avoided by removing the comb after the gel has been placed in the running buffer.

- Use TAE buffer for most molecular biology agarose gel electrophoresis.

- Use TBE buffer for resolution of fragments smaller than 300 bp.

Recipe

1 X TAE Buffer
Agarose Gel Electrophoresis Buffer
40 mM Tris-Acetate pH 7.8
1 mM EDTA

1 X TBE
Agarose and Polyacrylamide Gel Electrophoresis Buffer
0.089 M Tris
0.089 M Boric Acid
0.002 M EDTA

TBE vs. TAE

Agarose gel electrophoretic resolution of DNA depends on the concentration of agarose and the ionic strength of electrode buffer. There is a choice of buffers; TBE and TAE (Tris-acetate EDTA). TAE is the most commonly used electrophoresis buffer for routine molecular biology work.

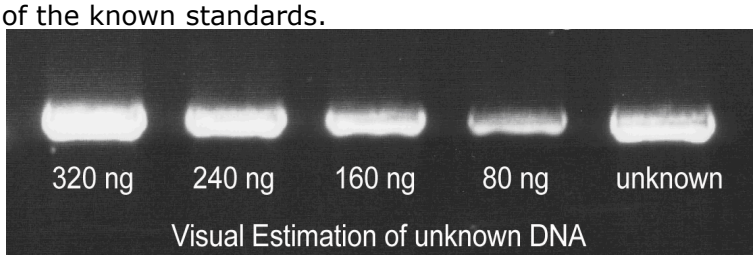
The resolution of supercoiled DNAs and large DNA is better in TAE than TBE. The buffering capacity of TAE is lower than TBE and is progressively depleted during successive electrophoresis. In contrast, TBE has a more stable and higher buffering capacity. Double stranded linear DNA fragments longer than ~500 bp migrate approximately 10 % faster in TAE than in TBE.

In summary, use TAE buffer for regular resolution of DNA fragments longer than ~500 bp but use TBE buffer for clear and higher resolution of smaller DNA fragments on agarose gels. Critical DNA sizes and gel concentrations for a clear separation were about 2-kb for the 0.8% agarose and 300-bp for the 2.0% agarose. DNA fragments larger than the critical size (>2-kb on 0.8% agarose gel) migrate faster in TAE, and the smaller fragments (<300-bp on 2% agarose gel) migrate faster in TBE showing better resolution.

Spectrophotometric Determination of DNA Concentration & Estimation by Agarose Gel Electrophoresis

Measuring the optical density (OD) or absorbance at 260 nm (A_{260}) in a UV spectrophotometer is a relatively accurate method for calculating the concentration of DNA in an aqueous solution if a standard curve is meticulously prepared. An A_{260} of 1, using a 1 cm path length, corresponds to a DNA concentration of 50 µg/ml for double stranded DNA, 40 µg/ml for RNA and 33 µg/ml for oligonucleotides. However, this method is not suitable for determining concentrations of dilute solutions of DNA, as the sensitivity of this method is not very high. For reliable readings, the concentration of double stranded DNA must be greater than 1 µg/ml. A simple, inexpensive method for the estimation of nanogram quantities of DNA is described in the following section. We recommend the use of agarose gel electrophoresis for routine approximate determination of DNA concentration.

The amount of DNA in a sample may be estimated by running the sample alongside standards containing known amounts of the same-sized DNA fragment. In the presence of ethidium bromide staining, the amount of sample DNA can be visually estimated by comparing the band intensity with that of the known standards.



Visual Estimation of unknown DNA

An unknown amount of a 4 kb DNA fragment (unknown) was run alongside known quantities (indicated in nanograms) of the same DNA fragment. As estimated by visual comparison with the known standards, the unknown sample contained 240-320 ng of DNA.

⚠ Ethidium bromide is a carcinogen. Follow Health and Safety Procedures established by your institution.
Follow proper Hazardous Material Disposal procedures established by your institution.

ⓘ • Use 0.1 µg of ethidium bromide solution for each ml of gel volume.

Ordering Information

Agarose Ultra Pure Molecular Biology Grade

Product	Catalog No.	Size*	Price $
Agarose LE Molecular Biology Grade; 100 gms	40-3010-10	100 gms	120.00
Agarose LE Molecular Biology Grade; 500 gms	40-3010-50	500 gms	410.00
Agarose LE Molecular Biology Grade; 1 KG	40-3010-01	1 KG	738.00
Agarose Tablets, 0.5 gm each	40-3011-10	100 tablets	100.00
Agarose HiRes Ultra Pure Molecular Biology Grade; 100 gms	40-3015-10	100 gms	206.00
Agarose HiRes Ultra Pure Molecular Biology Grade; 500 gms	40-3015-50	500 gms	985.00
Agarose HiRes Ultra Pure Molecular Biology Grade; 1 KG	40-3015-01	1 KG	1785.00
Agarose Low Melt Ultra Pure Molecular Biology Grade; 100 gms	40-3016-10	100 gms	285.00
Agarose Low Melt Ultra Pure Molecular Biology Grade; 500 gms	40-3016-50	500 gms	1140.00
Agarose Low Melt Ultra Pure Molecular Biology Grade; 1 KG	40-3016-01	1 KG	2166.00

Related Products Ordering Information

Bacteriological Growth Media

Product	Catalog No.	Size	Price $
Agar Type A Bacterial Culture Grade; 100 gms	40-3301-10	100 gms	18.00
Agar Type A Bacterial Culture Grade; 500 gms	40-3301-50	500 gms	81.00
Agar Type A Bacterial Culture Grade; 1 KG	40-3301-10	1 KG	145.80
Casein Peptone (Type 1) Bacterial Culture Grade; 100 gms	40-3305-10	100 gms	17.50
Casein Peptone (Type 1) Bacterial Culture Grade; 500 gms	40-3305-50	500 gms	78.80
Casein Peptone(Type 1) Bacterial Culture Grade; 1 KG	40-3305-01	1 KG	141.40
Yeast Extract Bacterial Culture Grade; 100 gms	40-3311-10	100 gms	11.25
Yeast Extract Bacterial Culture Grade; 500 gms	40-3311-50	500 gms	50.20
Yeast Extract Bacterial Culture Grade; 1 KG	40-3311-01	1 KG	90.60

Loading Buffers

Product	Catalog No.	Size	Price $
Loading Buffer 5X BPB/XC non-denaturing	40-3002-01	100 µl	5.00
Loading Buffer 5X BPB/XC non-denaturing	40-3002-10	1 ml	10.00
Loading Buffer 5X Orange G/XC non-denaturing	40-3004-01	100 µl	5.00
Loading Buffer 5X Orange G/XC non-denaturing	40-3004-10	1 ml	10.00
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-01	100 µl	5.00
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-10	1 ml	10.00

Buffers& Reagents

Product	Catalog No.	Size	Price $
TAE Buffer; 50 X Concentrate	40-3007-01	100 ml	32.00
TBE Buffer; 5 X Concentrate	40-3008-10	1000 ml	35.00
Hybwash A, Hybridization Wash Solution	40-5020-20	200 ml	65.00
Hybwash B, Hybridization Wash Solution	40-5021-10	100 ml	50.00
10x Washing buffer	40-5025-20	200 ml	125.00
10% Blocking solution	40-5026-10	100 ml	75.00
Seq. Loading buffer	40-5027-00	1 ml	10.00
10x AP Detection buffer	40-5031-10	100 ml	65.00
Lumisol™ I Hybridization Solution; contains formamide	40-5022-20	200 ml	75.00
Lumisol™ II Hybridization Solution; for non-toxic hybridizations	40-5023-20	200 ml	75.00
Lumisol™ III Hybridization Solution; for oligo probes	40-5024-20	200 ml	75.00

Omni-Marker™

Product	Catalog No.	Size*	Price $
Omni-Marker™ Universal unlabeled	40-3005-01	100 µl	15.00
Omni- Marker™ Universal unlabeled	40-3005-05	500 µl	50.00
Omni-Marker™ Universal unlabeled	40-3005-10	1 ml	90.00
Omni- Marker™ Low unlabeled	40-3006-01	100 µl	15.00
Omni-Marker™ Low unlabeled	40-3006-05	500 µl	50.00
Omni- Marker™ Low unlabeled	40-3006-10	1 ml	90.00
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	40-3062-01	100 µl	75.00
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	40-3062-05	500 µl	325.00

Loading Buffers

Product	Catalog No.	Size	Price $
Loading Buffer 5X BPB/XC non-denaturing	40-3002-01	100 µl	5.00
Loading Buffer 5X BPB/XC non-denaturing	40-3002-10	1 ml	10.00
Loading Buffer 5X Orange G/XC non-denaturing	40-3004-01	100 µl	5.00
Loading Buffer 5X Orange G/XC non-denaturing	40-3004-10	1 ml	10.00
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-01	100 µl	5.00
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-10	1 ml	10.00

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