Product Specifications

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

TAE Buffer; 50X Concentrate

Store at Room Temperature

	Catalog Number	Description	Size
❑	40-3007-01	TAE Buffer; 50X Concentrate	100 mL
❑	40-3007-05	TAE Buffer; 50X Concentrate	500 mL
❑	40-3007-10	TAE Buffer; 50X Concentrate	1 L

Product Description & Application

50X TAE (Tris-Acetate-EDTA) buffer solution consists of 2.0M Tris and 0.05M EDTA adjusted to pH 7.8 (± 0.2) using glacial acetic acid. Dilute to 1X using sterile water for a solution with a final composition of 40mM Tris Acetate, pH 7.8 (± 0.2) and 1.0mM EDTA. Dilution to 0.5 X concentration also has been shown to achieve comparable electrophoretic separation. At Gene Link we use 0.5X concentration and recommend it.

TAE vs. TBE

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; TAE and TBE (Tris-borate 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.

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

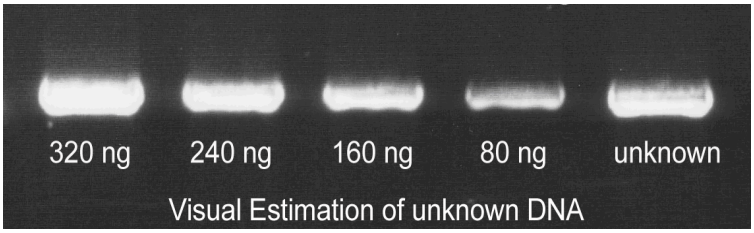
1 X TAE Buffer
Agarose Gel Electrophoresis Buffer
40 mM Tris-Acetate pH 7.8
1 mM EDTA

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

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.

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Gene Link™

Ordering Information

Southern Blot Buffers& Reagents

Product	Catalog No.	Unit Size
Agarose Tablets, 0.5 gm each	40-3011-10	100 tablets
Agarose LE Molecular Biology Grade; 100 gms	40-3010-10	100 gms
Agarose LE Molecular Biology Grade; 500 gms	40-3010-50	500 gms
Hybwash A, Hybridization Wash Solution (20X SSC)	40-5020-20	200 mL
Hybwash B, Hybridization Wash Solution (10% SDS)	40-5021-10	100 mL
TAE Buffer; 50 X Concentrate; 100 ml	40-3007-01	100 mL
TAE Buffer; 50 X Concentrate; 1000 ml	40-3007-10	1 L
TBE Buffer; 5 X Concentrate	40-3008-10	1 L
Maleic acid buffer 10X (Buffer M 10X)	40-5025-20	200 mL
10% Blocking solution	40-5026-10	100 mL
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-10	1 mL
10x AP Detection buffer (alkaline phosphatase detection buffer)	40-5031-10	100 mL
Lumisol™ I Hybridization Solution; contains formamide	40-5022-20	200 mL
Lumisol™ II Hybridization Solution; for non-toxic hybridizations	40-5023-20	200 mL
Lumisol™ III Hybridization Solution; for oligo probes	40-5024-20	200 mL
CDP-Star® Substrate; Ready-to-Use 0.25 mM in spray bottle; 10 mL	40-5010-10	10 mL
Depurination Solution (2X) for Southern Blotting; 1 L	40-5034-10	1 L
Denaturation Solution (2X) for Southern Blotting; 1L	40-5035-10	1 L
Neutralization Solution (2X) for Southern Blotting; 1L	40-5036-10	1 L

Loading Buffers

Product	Catalog No.	Size
Gel Loading Buffer 5X BPB/XC non-denaturing	40-3002-10	1 mL
Gel Loading Buffer 5X BPB/XC non-denaturing	40-3002-15	15 mL
Gel Loading Buffer 10X BPB/XC non-denaturing	40-3003-10	1 mL
Gel Loading Buffer 10X BPB/XC non-denaturing	40-3003-15	15 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing	40-3004-10	1 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing	40-3004-15	15 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-10	1 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-15	15 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer	40-5028-10	1 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer	40-5028-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide	40-5029-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide	40-5029-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide	40-5030-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide	40-5030-15	15 mL

Related Products Ordering Information

Omni-Pure™ Plasmid DNA Purification Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Pure™ Plasmid DNA Purification System	40-4020-01	100
Omni-Pure™ Plasmid DNA Purification System	40-4020-05	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Clean™ Gel DNA Purification and Concentration Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Clean™ Gel DNA Beads Purification System	40-4110-10	100
Omni-Clean™ Gel DNA Beads Purification System	40-4110-50	500
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-10	100
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-50	500
Omni-Clean™ DNA Beads Concentration System	40-4130-10	100
Omni-Clean™ DNA Beads Concentration System	40-4130-50	500
Omni-Clean™ DNA Spin Column Concentration System	40-4140-10	100
Omni-Clean™ DNA Spin Column Concentration System	40-4140-50	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Pure™ DNA & RNA Purification Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Pure™ Blood DNA Purification System	40-4010-01	100
Omni-Pure™ Blood DNA Purification System	40-4010-05	500
Omni-Pure™ Blood DNA Purification System	40-4010-10	1000
Omni-Pure™ Tissue DNA Purification System	40-4050-01	100
Omni-Pure™ Tissue DNA Purification System	40-4050-05	500
Omni-Pure™ Tissue DNA Purification System	40-4050-10	1000
Omni-Pure™ Plant DNA Purification System	40-4060-01	100
Omni-Pure™ Plant DNA Purification System	40-4060-05	500
Omni-Pure™ Plant DNA Purification System	40-4060-10	1000
Omni-Pure™ Viral DNA Purification System	40-3720-01	100
Omni-Pure™ Viral DNA Purification System	40-3720-05	500
Omni-Pure™ Microbial DNA Purification System	40-3700-01	100
Omni-Pure™ Microbial DNA Purification System	40-3700-05	500
Omni-Pure™ Viral RNA Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Purification System	40-3650-05	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

All Gene Link products are for research use only Current pricing are posted at http://www.genelink.com/

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