Product Specifications

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

TBE Buffer; 5X Concentrate

Store at Room Temperature

	Catalog Number	Description	Size
❑	40-3008-10	TBE Buffer; 5X Concentrate	1000 ml

Product Description & Application

TBE (Tris-Borate-EDTA) 5X buffer concentrate solution consists of 0.45M Tris, 0.45M Borate and 0.01M EDTA adjusted to pH 8.0 (± 0.2). Dilute to 1X using sterile water for use as electrophoresis buffer. Dilution to 0.5X concentration also has been shown to achieve comparable electrophoretic separation. At Gene Link we use 0.5X concentration and recommend it.

Concentrated TBE buffers are prone to precipitation. They are shown to still retain buffering capacity and can be put to limited use instead of discarding.

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.

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

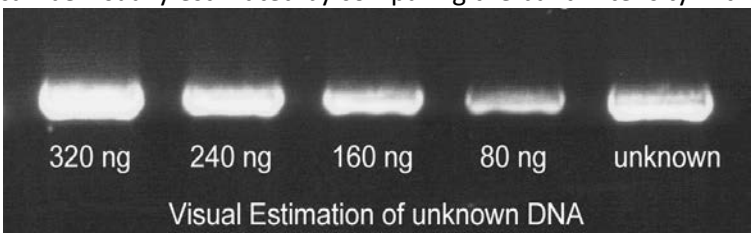
1 X TAE Buffer
Agarose Gel Electrophoresis Buffer
40 mM Tris-Acetate pH 7.8
1 mM EDTA

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.

Ordering Information

Product	Catalog No.	Size*
TAE Buffer; 50 X Concentrate	40-3007-01	100 mL
TAE Buffer; 50 X Concentrate	40-3007-05	500 mL
TBE Buffer; 5 X Concentrate	40-3008-10	1000 mL

Related Products Ordering Information

Buffers& Reagents

Product	Catalog No.	Size
Agarose Tablets, 0.5 gm each	40-3011-10	100 tablets
Agarose LE Molecular Biology Grade	40-3010-10	100 gms
Agarose LE Molecular Biology Grade	40-3010-50	500 gms
Hybwash A, Hybridization Wash Solution	40-5020-20	200 mL
Hybwash B, Hybridization Wash Solution	40-5021-10	100 mL
10x Washing buffer	40-5025-20	200 mL
10% Blocking solution	40-5026-10	100 mL
Seq. Loading buffer	40-5027-00	1 mL
10x AP 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

Omni-Marker™

Product	Catalog No.	Size*
Omni-Marker™ Universal unlabeled	40-3005-01	100 µL
Omni- Marker™ Universal unlabeled	40-3005-05	500 µL
Omni-Marker™ Universal unlabeled	40-3005-10	1 mL
Omni- Marker™ Low unlabeled	40-3006-01	100 µL
Omni-Marker™ Low unlabeled	40-3006-05	500 µL
Omni- Marker™ Low unlabeled	40-3006-10	1 mL
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	40-3062-01	100 µL
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	40-3062-05	500 µL

Loading Buffers		
Product	**Catalog No.**	Size
Loading Buffer 5X BPB/XC non-denaturing	40-3002-01	100 µL
Loading Buffer 5X BPB/XC non-denaturing	40-3002-10	1 mL
Loading Buffer 5X Orange G/XC non-denaturing	40-3004-01	100 µL
Loading Buffer 5X Orange G/XC non-denaturing	40-3004-10	1 mL
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-01	100 µL
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-10	1 mL

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