ProductInformation

PAGE SOLUTION For DNA Sequencing
8% acrylamide/bis-acrylamide (19:1) solution

Product No. **P 8329**
Store at 2-8°C

Product Description

Denaturing polyacrylamide-urea gels have been used extensively for DNA sequencing and are also useful in separating small RNA transcripts or mRNA. An 8% polyacrylamide gel is capable of sequencing from 25 to 400 nucleotides from the terminus of the primer in dideoxy sequencing or to within 50 nucleotides of the labeled terminus in Maxam-Gilbert sequencing. Many factors influence the number of nucleotides that can actually be sequenced such as the run time, gel thickness (0.2 to 0.6 mm), and gel height (40 to 100 cm). Gel thickness influences resolution and electrophoretic mobility. Thin gels (0.2 mm) give the best resolution but are fragile; thick gels (0.6 mm) allow larger sample volumes to be loaded but are difficult to dry and fix. Most commonly used are 0.4 mm gels of constant thickness. Wedge shaped gels (thin at the top, thick at the bottom) are sometimes used to increase the amount of readable sequence. The thicker, lower resistance cross-section at the bottom of the gel allows the smaller fragments to slow down compared to the larger fragments at the top of the gel.

Reagents Required But Not Provided
(Sigma Product Numbers have been given where appropriate)

- 10% (w/v) Ammonium persulfate, Product No. A9164, bulk, or A3426, preweighed 150 mg capsules. Prepare solution immediately before use.
- TEMED, Product No. T7024

Procedure

Assemble the glass plates and spacers as indicated by the gel sequencing apparatus instructions. Add 0.6 ml of 10% (w/v) ammonium persulfate (prepared immediately before use) and 45 µl TEMED directly to the 100 ml bottle of PAGE solution. Mix well and immediately pour between the glass plates of the sequencing apparatus. If necessary, remove any trapped air bubbles by gently tapping on the glass plates. Follow sequencing apparatus instructions for inserting combs. Allow gel to polymerize approximately 1 hour at room temperature. Schlieren lines just under the combs are a good indication that polymerization is complete.

Product Profile

Composition:

8% (w/v) acrylamide/bis-acrylamide (19:1)
100 mM Tris-borate pH 8.3
1 mM EDTA
7 M Urea

Suitable for DNA sequencing
DNase, RNase, Protease: None detected

References

1. Sambrook, J., *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory (1989). p. 13.45-13.101
2. Ausubel, F.M., *et al.*, Current Protocols in Molecular Biology, John Wiley and Sons, Inc., (1994). p. 7.1-7.7
3. Sanger, F., *et al.*, Proc. Nat. Acad. Sci. USA, **74**, 5463 (1977)
4. Maxam, A.M. and Gilbert, W., Meth. Enzymol., **65**, 499 (1980)

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Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply.
Please see reverse side of the invoice or packing slip.