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Roche

Agarose LE (low electroendosmosis)

For use in standard gel electrophoresis

Cat. No. 11 685 660 001 100 g
Cat. No. 11 685 678 001 500 g

Version 08
Content version: December 2009
Store at +15 to +25°C

1. What this Product Does

Properties

Agarose LE is suitable for analytical and preparative electrophoresis of nucleic acids in standard agarose gels. The appropriate size range of nucleic acid separation with Agarose LE is between 0.2–15 kbp depending on the concentration of Agarose LE applied.

LE is tested for preparative electrophoresis and isolation of DNA fragments.

Specifications

Electroendosmosis (EEO)	0.05 - 0.13
Sulfur as SO_4	≤ 0.14%
Gelling temperature (1.5 %)	+36°C (± 1.5°C)
Melting temperature (1.5 %)	+88°C (± 1.5°C)
Gel strength (1%)	≥ 1,200 g/cm^2
Gel strength (1.5 %)	≥ 2,500 g/cm^2
DNase	none detected
RNase	none detected

Digestion of electroeluted DNA is tested using the restriction endonucleases *Bam* HI and *Pst* I.

Recovered DNA can be ligated with T4 DNA ligase.

Application

Agarose LE can be used for

- the analysis of PCR products,
- examination of restriction endonucleases,
- digests of plasmid, cosmid and λ phage DNA and
- electrophoresis of RNA in *e.g.* denaturing gels containing formaldehyde.

Nucleic acid fragments separated with Agarose LE can be blotted to nylon or nitro-cellulose membranes by all standard blotting techniques.

⚠ Detection with non-radioactive probes, *e.g.* digoxigenin (DIG)-labeled nucleic acids, does not interfere with the use of Agarose LE.

Quality Control

Agarose LE is tested:

- in the analytical electrophoresis of DNA of various length,
- in Southern blots,
- in separation of RNA and subsequent Northern blotting,
- in preparative electrophoresis of DNA and
- in isolation of DNA fragments followed by restriction digests and ligation.

Storage and Stabilty

Agarose LE should be stored cool and dry at +15 to +25°C until the expiration date printed on the label.

2. Preparation of Agarose Gels

Protocol

Please refer to the following table:

Step	Action
❶	Use a flask that is 2 to 4 times the volume of the solution being prepared.
❷	Add the correct amount of dry agarose to a measured quantity of electrophoresis buffer.
❸	If you use a **boiling wather bath**: • melt the agarose, simply by heating the slurry in a boiling water bath until the agarose dissolves. If you use **a microwave oven:** • Heat the slurry in a microwave oven on a high power setting until it starts to boil. • Allow the solution to boil for 1 min or until all particles are dissolved. • Remove the flask from the microwave oven, and gently swirl to mix the agarose solution. ⚠ Use extreme caution when handling. The solution may become superheated and boil vigorously when touched.
❹	Cool the solution to approx. 60°C before pouring.

Electrophoresis of DNA and RNA (1,2)

The most commonly used technique for DNA separation is electrophoresis in horizontal agarose gels submerged in either Tris-acetate or Tris-borate buffer. RNA molecules are separated in denaturing agarose gels containing formaldehyde. RNA electrophoresis is performed in MOPS buffer.

The efficient separation of DNA fragments of a wide size range is possible by adjusting the agarose concentration accordingly. The resolution ranges which can be obtained with various concentrations of Agarose LE are shown in the table together with the size of DNA fragments which comigrate with bromphenol blue which is often used as a dye to monitor the extend of electrophoresis.

Concentration of Agarose LE in gel (%)	Efficient range of separation of linear DNA molecules (kbp)	Size of linear DNA fragment that co-migrate with bromphenol blue (bp)
0.8	1–15	950
1	0.5–10	525
1.25	0.3–5	450
1.5	0.2–4	400
1.75	0.2–2.5	300

Staining DNA in Agarose Gels

The most common stain for detecting nucleic acids in agarose gels is ethidium bromide. It can be used in a concentration range between 0.5 and 1 μg/ml directly in the gel and in the electrophoresis buffer.

⚠ If the gel contains more than 5 μg/ml, it is not necessary to add ethidium bromide to the running buffer.

Changes to previous Version

Update of Regulatory Disclaimer

Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products e.g., enzymes, agaroses and molecular weight markers or manuals, please visit and bookmark our homepage http://www.roche-applied-science.com:

References

1 Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edition, CSH Laboratory Press, Cold Spring Harbor, New York

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