Agarose NA

Data File

Submarine system

Amersham Biosciences Agarose NA is designed specifically for use in the electrophoresis of nucleic acids.

- Reliable separation of nucleic acids
- Low inhibition of enzyme activity on recovered material
- High gel strength

Properties

Agarose gel electrophoresis is a standard technique for the separation of DNA and RNA molecules. It is used for both analytical and preparative purposes. The technique is simple to perform and capable of resolution unequalled by alternative procedures.

All agarose types are not the same. A number of physical and functional properties may be defined which determine whether or not a praticular agarose is suitable for nucleic acid applications.

Amersham Biosciences Agarose NA has been perpared to give the optimal combination of these properties.

Physical properties

Agarose gels contain positively charged ions which are free to move in an electric field. When a potential is applied, these ions, along with their associated water of hydration, migrate towards the cathode and a liquid flow is set up. This cathodal flow of liquid, termed electroendosmosis (EEO), is opposite in direction to the migration of nucleic acid molecules during electrophoresis and disrupts their separation. An agarose which shows minimal EEO is therefore optimal for separation.

Agarose NA is a low electroendosmosis material ($-m = 0.10 \pm 0.01$) which produces gels with excellent nucleic acid separation properties.

The running and processing of agarose gels involves a number of steps in which manual handling is required. The physical strength of the gel is therefore important, expecially where gels of low agarose content are concerned. Agarose NA produces gels of high strength (1.5% gel >2400 g/cm^2) which are easy to handle even at low agarose percentages.

Vizualization of small amounts of nucleic acid in agarose gels may be hampered by background absorbance by the gel matrix. Agarose NA produces solutions and gels of high optical clarity. This helps to ensure maximum sensitivity of detection of DNA and RNA.

Functional properties

Agarose for nucleic acid electrophoresis should allow separation and recovery of undamaged and uncontaiminated material. Some agaroses contain traces of exo-and/or endonucleases which damage nucleic acids, giving unreliable separations and uncertain reactivity of recovered material. Nucleic acid molecules recovered from agarose gels often contain contaminants which inihibit subsequent enymic reactions. This inhibition is thought to be due, at least partially, to the presence of sulphate containing agaropectin molecules.

Amersham Biosciences Agarose NA is function tested for quality of separation and purity of recovered material.

- Restriction fragments of pBR 322 DNA are separated by electrophoresis on Agarose NA gels and the gels are scanned with a microdensitometer. The gel material is acceptable only if the densitometer trace returns to baseline between peaks, indicating no exonuclease damage to the sample.

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- Fragments of pBR 322 DNA are extracted from gels by electroelution. Recovered material is then tested for its activity as a substrate both for a series of restriction enzymes and for T4 DNA ligase. The activity level of extracted fragments is compared with that of similar DNA which has not been separated on a gel. Only agarose batches which show very high relative activity with all enzymes are acceptable as Agarose NA.
- A high degree of reactivity of recovered material with both restriction enzymes and T4 DNA ligase indicates the absence from Agarose NA of enzyme inhibitors. Reactivity as a substrate for DNA ligase also indicates that the termini of recovered fragments are substantially undamaged and hence that exonuclease activity in Agarose NA is low.

Details of function testing data for individual batches are found on the Certificate of Analysis which accompanies each package of Agarose NA.

Applications

The properties described above demonstrate that Amersham Biosciences Agarose NA is an ideal matrix for gel electrophoresis of nucleic acids. Agarose NA is suitable for all analytical separations as well as most preparative methods.

Specifications

Agarose NA	
Gelling temperature (2% soln.)	34–37 °C
Gel strength (1.5% gel)	2 400 g/cm^2
Optical clarity (530 nm, 1.5% soln., 90 °C	>99% T
Electroendosmosis (-mr)	0.10
Sulphate	<0.08–0.12
Moisture content	<7.0%

Ordering Information

	Pack Size	Code No.
Agarose NA	10 g	17-0554-01
	100 g	17-0554-02
	1 kg	17-0554-03

Related products

	Pack Size	Code No.
Ethidium bromide	10 ml	17-1328-01
Bromphenol Blue	10 g	17-1329-01
Tris	500 g	17-1321-01
EDTA	100 g	17-1324-01
Boric acid	500 g	17-1322-01
Ficoll 400	100 g	17-0400-01
DRIgest III DNA-H*ind* III/ϕX-174 RF DNA-H*ae* III Digest	25 µg	27-4060-01
λ DNA-H*ind* III/ϕX-174 RF DNA-H*inc* II Digest	25 µg	27-4052-01
λ DNA-H*ind* III/ϕX-174 RF DNA-H*ae* III Digest	25 µg	27-4054-01
λ DNA-H*ind* III Digest	100 µg	27-4048-01
ϕX-174 RF DNA-H*inc* II Digest	10 µg	27-4040-01
ϕX-174 RF DNA-H*ae* III Digest	10 µg	27-4044-01

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