Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Top Vision™ Low Melting Point Agarose

#R0801 25 g

Lot: _

Store at room temperature

ISO 9001 ISO 14001
www.fermentas.com

Description

Top Vision™ Low Melting Point Agarose is highly purified agarose with a low melting temperature certified by strict quality control test procedures.

Characteristics

Electroendosmosis (EEO)	0.11
Gel strength (1% gel)	355 g/cm^2
Gel strength (1.5% gel)	1095 g/cm^2
Gel point (1.5% gel)	27.0°C
Melting point (1.5% gel)	64.8°C
Moisture	3.01%

QUALITY CONTROL

No detectable RNase and DNase activity.

Quality authorized by: Jurgita Zilinskiene

Applications

- Preparation of DNA preparative gels in routine molecular biology techniques.
- In-gel enzymatic processing (digestion, ligation etc.).
- The DNA recovered from agarose gels after electrophoresis can be used in enzymatic process (restriction, ligation, etc.) (1).
- Digestion of remelted agarose by agarase enzymes makes it very easy to recover large DNA fragments suitable for cloning or enzymatic processing.
- May be used at concentrations between 0.8-2.0% with typical buffer systems.

Protocol for recovery of DNA from Top Vision™ Low Melting Point Agarose gels using Fermentas' Agarase (#E00461)

I. AGAROSE DIGESTION

1. Perform electrophoresis of DNA in a low melting agarose gel prepared in TAE, 0.5X TBE, TBE or TPE buffer. Stain the gel with ethidium bromide.
2. Cut out the desired band from the agarose gel with a clean, nuclease free spatula. Limit UV exposure of the gel slice to a minimum. Cut out as much agarose as is necessary to recover the DNA band.
3. Place the gel slice into a pre-weighed 1.5 ml microcentrifuge tube and determine the weight of the slice. To facilitate melting, cut gel slices larger than 200 mg into smaller pieces.
4. Incubate the tube for approx. 10 min at 70°C until the agarose is **completely** melted.

Note

- Incubation at elevated temperatures may denature DNA.
- Ensure that the gel slice is thoroughly melted. If the agarose is not completely melted, the hydrolysis also will be incomplete.

5. Transfer the tube to a 42°C water bath and equilibrate for 5 min prior to adding Agarase.
6. Add 1 unit of Agarase per 100 mg (approx. 100 µl) of 1% agarose; gently mix and incubate for 30 min at 42°C.

Note

- If you are using a higher percentage agarose, the amount of Agarase should be proportionately increased.

II. DNA PURIFICATION

DNA fragments larger than 30 kb

Large DNA fragments require delicate handling to avoid mechanical shearing.

1. Centrifuge at 15000 x g for 10 min to pellet undigested carbohydrates.
2. Remove oligosaccharides and Agarase by dialysis or carry out subsequent manipulations with DNA in the digested agarose solution.

DNA fragments smaller than 30 kb

1. Add salt to hydrolyzed agarose: ammonium acetate to 2.5 M or sodium acetate to 0.3 M.

Note

- Use ammonium acetate rather than other salts, because they may cause co-precipitation of oligosaccharides with DNA.
- T4 polynucleotide kinase is inhibited by ammonium ions. Use sodium acetate if, following recovery, you will be labeling 5'-ends of DNA with T4 polynucleotide kinase.

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2. Chill on ice for 5 min, centrifuge at 15000 x g for 10 min to pellet undigested carbohydrates.
3. Transfer the supernatant to a clean tube. Add 1 volume of isopropanol or 2-3 volumes of ethanol, mix gently and incubate at least for 30 min at 0°C to 22°C.

Note

- If DNA fragments are <500 bp or if DNA concentration is <0.05 µg/ml, incubate overnight at 0°C to 22°C.

4. Centrifuge at 15000 x g for 15 min, remove supernatant and dry pellet. The pellet can be resuspended in an appropriate buffer for subsequent manipulation.

Reference

1. Current Protocols in Molecular Biology (Ausubel, F.M., ed.), John Wiley & Sons, Inc., Brooklyn, New York, 2.6.5-2.6.7, 1999.

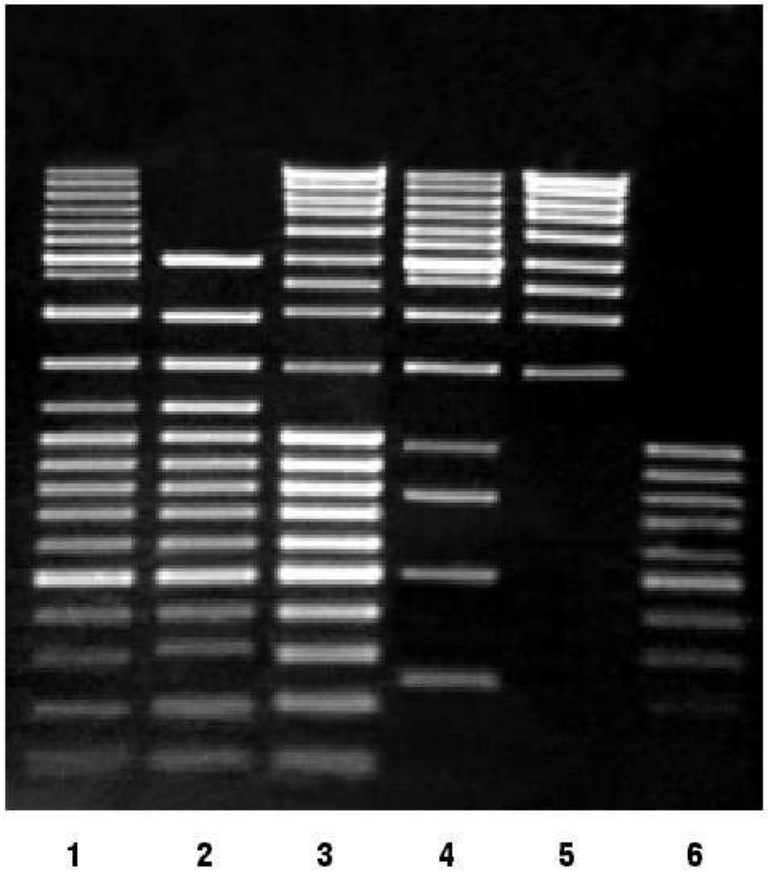

Fig. 1. Electrophoresis of Fermentas' DNA Size Markers. 1% Top Vision™ Low Melting Point Agarose, 1X TAE.

1 – GeneRuler™ DNA Ladder Mix
2 – GeneRuler™ 100 bp DNA Ladder Plus
3 – GeneRuler™ DNA Ladder Mix, ready-to-use
4 – GeneRuler™ 1 kb DNA Ladder
5 – MassRuler™ DNA Ladder, High Range, ready-to-use
6 – GeneRuler™ 100 bp DNA Ladder