Agarose Gel Separation of Reovirus Particles by sigma1 Content

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Abstract

Separation of reovirus particles by gel electrophoresis

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Before start

Materials and Reagents

-1M Tris Acetate pH 7.2

- MW = 121.1g/mol
- Add 60.5 g Tris base to 300 ml ddH_2O
- pH to 7.2 with glacial acetic acid
- Fill to 500 ml total volume

-10X TAE pH 7.2

- 125 ml 1M Tris acetate pH 7.2
- 50 ml EDTA pH 8.0
- 75 ml ddH_2O
- Dilute to 1x as needed and filter 0.45 uM to remove any potential debris

-UltraPure™ Agarose, Thermo Scientific

-NOVEX® Colloidal Blue Staining Kit (follow directions for [high] Tris Glycine)

-Reovirus 2x Loading Dye

- 5% Ficoll 400 (0.5g)

- 05% Bromophenol blue (50 mg)
- 2 ml 10X TAE pH 7.2
- Fill to 10 ml with ddH_2O

Protocol

Step 1.
Combine 0.4 g of agarose with 40 ml of TAE

Step 2.
Microwave until agarose is thoroughly melted

Step 3.
Add water to replace water lost during boiling up to 40 ml and mix well

Step 4.
Pour 35 ml agarose to clean casting rig (7x10 cm)

Step 5.

Mix purified reovirus and loading dye 1:1 at 1x1011 particles per well (25 ul max)

Step 6.
Run at constant 25V for 18 h

Step 7.
Stain gel with colloidal blue as directed

Step 8.
Destain gel in water overnight

Step 9.
Image gel by on Licor Odyssey Imaging System