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Agarose Gel Separation of Reovirus Particles by sigma1 Content

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Protocol status: Working

ABSTRACT

Separation of reovirus particles by gel electrophoresis

ATTACHMENTS

[Sigma 1 Particle Gel.docx](#)

BEFORE START INSTRUCTIONS

Materials and Reagents

- 1M Tris Acetate pH 7.2
 - MW = 121.1g/mol
 - Add 60.5 g Tris base to 300 ml ddH₂O
 - 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 ddH₂O
 - Dilute to 1x as needed and filter 0.45 µm 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 ddH₂O

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attachment fiber

- 1 Combine 0.4 g of agarose with 40 ml of TAE
- 2 Microwave until agarose is thoroughly melted
- 3 Add water to replace water lost during boiling up to 40 ml and mix well
- 4 Pour 35 ml agarose to clean casting rig (7x10 cm)
- 5 Mix purified reovirus and loading dye 1:1 at 1×10^{11} particles per well (25 ul max)
- 6 Run at constant 25V for 18 h
- 7 Stain gel with colloidal blue as directed

8 Destain gel in water overnight

9 Image gel by on Licor Odyssey Imaging System