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Protocol status: Working

Fluorescent Labeling of Reovirus with Succinimidyl Ester Dyes V.2

PLOS Pathogens

Bernardo Mainou¹

¹Emory University

Bernardo Mainou
Emory University

ABSTRACT

Fluorescent labeling of reovirus virions with succinimidyl ester-conjugated fluorescent probes

ATTACHMENTS

[Reovirus succinimidyl ester dye labeling - BAM.docx](#)

BEFORE START INSTRUCTIONS

You will need purified reovirus and dimethylformamide. Sodium bicarbonate must be made on the day of labeling. SE-conjugated dyes are not limited to Alexa dyes.

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- 1** Dilute 3×10^{12} reovirus particles in up to 495 μ l of fresh 0.05 M sodium bicarbonate (pH 8.5)
 - a. Sodium Bicarbonate (FW = 84.01)
 - b. Make a 10X Stock (0.5 M) in ddH₂O (0.42 g into 10 ml)
 - c. Make a 1X working solution (0.05 M) in ddH₂O.Filter Sterilize with a 0.2 μ m filter
- 2** Make a 1 mM stock of the succinimidyl ester Alexa fluor in dimethylformamide.
 - a. For Alexa 546 (MW = 1159.6) (e.g. 862 μ l dimethylformamide into 1 mg of dye)
- 3** Add 5 μ l of 1 mM Alexa dye to virus-sodium bicarbonate solution
 - a. In effect, you are labeling the virus with 10 μ M of dye
- 4** Rock for 90 minutes in the **dark** at room temperature or 4°C (we have not observed significant differences between labeling at either temperature, but 4°C may enhance stability of virus)
- 5** Dialyze (6-8 kDa molecular weight cut-off) in PBS overnight at 4°C, replacing with the PBS after the first hour of dialysis
- 6** Store virus at 4°C

Note: for a Mock control, simply follow the above steps without including viral particles. In other words, add 5 μ l of 1 mM dye to 500 μ l of 0.05 M sodium bicarbonate and proceed with the labeling protocol.