Fluorescent Labeling of Reovirus with Succinimidyl Ester Dyes Version 2

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Abstract

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

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Before start

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.

Protocol

Step 1.

Dilute 3e12 reovirus particles in up to 495 ul 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 ddH20 (0.42 g into 10 ml)

c. Make a 1X working solution (0.05 M) in ddH20d.

Filter Sterilize with a 0.2 um filter

Step 2.

Make a 1 mM stock of the succinimidyl ester Alexa fluor in dimethylformamidea.

a. For Alexa 546 (MW = 1159.6) (e.g. 862 ul dimethylformamide into 1 mg of dye)

Step 3.

Add 5 ul of 1 mM Alexa dye to virus-sodium bicarbonate solution

a. In effect, you are labeling the virus with 10 uM of dye

Step 4.

Rock for 90 minutes in the **dark** at room temperature or 4C (we have not observed significant differences between labeling at either temperature, but 4C may enhance stability of virus)

Step 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

Step 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 ul of 1 mM dye to 500 uL of 0.05 M sodium bicarbonate and proceed with the labeling protocol.