Fluorescent Staining of T5-phages

Steven Wilhelm

Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) for additional information regarding this protocol.

Citation: Steven Wilhelm Fluorescent Staining of T5-phages. **protocols.io**
dx.doi.org/10.17504/protocols.io.iksccwe
Published: 24 Jun 2017

Protocol

Preparing T5 for Staining
Step 1.

Pellet phage in an ultracentrifuge at 28,000 rpm, 90 min, 10ºC in 35 mL polycarbonate tubes, filled to the rim, balanced with phage buffer so that the difference is <0.01 g.

NOTES
Alyssa Alsante 23 Jun 2017

Use the SW-28 rotor, or equivalent, cooled down in the fridge prior to using.

Preparing T5 for Staining
Step 2.

Discard the supernatant

Preparing T5 for Staining
Step 3.

Resuspend the pellet with phage buffer

AMOUNT
800 µl Additional info:

Preparing T5 for Staining
Step 4.

If the pellet still seems to have some cellular debris in it, filter the resuspension using a 1 mL syringe and a small 0.2 µm pore size syringe filter.

Preparing T5 for Staining
Step 5.

Re-pellet the viruses in a Beckmann TL-100 ultracentrifuge at 35,000 rpm, 2 hr, 6ºC using the TL-55 rotor cooled down befoer use using 1.4 mL polycarbonate tubes, filled and balanced as the bigger tubes in step 1.

Preparing T5 for Staining

Step 6.

Resuspend the pellet with phage buffer and transfer into 1.5 mL screw cap tubes

AMOUNT

100 µl Additional info:

Preparing T5 for Staining

Step 7.

Store at 4ºC

Staining T5 for Staining

Step 8.

Add YO-PRO stain to each tube containing 100 µL resuspension

AMOUNT

0.5 µl Additional info:

NOTES

Alyssa Alsante 23 Jun 2017

Make sure to work in the dark, because the stain is photosensitive.

Staining T5 for Staining

Step 9.

Cover the tubes with aluminum foil and let sit at 4ºC for 48 hrs

Staining T5 for Staining

Step 10.

After incubation, increase the volume to 1.4 mL

Staining T5 for Staining

Step 11.

Centrifuge in the Beckmann TL-100 as in step 5

Staining T5 for Staining

Step 12.

Discard the supernatant

Staining T5 for Staining

Step 13.

Suspend the viruses in Milli-Q water

AMOUNT
100 µl Additional info: