Propagating T5-phages for Fluorescent Staining

Dr. Steven Wilhelm

Abstract

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

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Protocol

Propagating T5-phages
Step 1.

Grow a culture of E.coli ATCC11303 in LB media overnight

Propagating T5-phages
Step 2.

The next morning, prewarm LB-agar plates to 37ºC

Propagating T5-phages
Step 3.

Pellet 1 mL of the cells in an Eppendorf tube at 10,000 xg, 2 min

Propagating T5-phages
Step 4.

Discard the medium

Propagating T5-phages
Step 5.

Resuspend the pellet into 1 mL 10 mM $MgSO_4$

AMOUNT
1 ml Additional info:
REAGENTS
Magnesium sulfate heptahydrate by Contributed by users

Propagating T5-phages

Step 6.

Melt 0.6% top agar in the microwave

Propagating T5-phages

Step 7.

Prepare sterile 5 mL tubes and pipet 3 mL of melted top agar into them

AMOUNT

3 ml Additional info:

Propagating T5-phages

Step 8.

Keep tubes in 45ºC heat block or water bath so that the agar stays in a liquid state, but not too hot to kill the cells.

Propagating T5-phages

Step 9.

Add 10 µL T5 from the stock (except the control) to 100 µL of resuspended cells

Propagating T5-phages

Step 10.

Incubate 5 min at room temperature

Propagating T5-phages

Step 11.

Add the infected cells to the molton top agar

Propagating T5-phages

Step 12.

Vortex briefly

Propagating T5-phages

Step 13.

Pour onto prewarmed LB agar plate and tilt the plate to spread

Propagating T5-phages

Step 14.

Let top agar solidify 30-60 min

Propagating T5-phages

Step 15.

Evert plates and return to 37ºC incubator

Propagating T5-phages

Step 16.

Let grow 6-8 hrs

Propagating T5-phages

Step 17.

When confluent plaques are seen on the plate, add 5 mL of sterile phage buffer (10 mM $MgSO_4$; 1 mM $CaCl_2$; 10 mM Tris-HCl, pH 7.5; 1% gelatin)

Propagating T5-phages

Step 18.

Let the plates sit overnight at 4ºC with the buffer on top to gather the phages

Propagating T5-phages

Step 19.

The next day, transfer the liquid from the plates to a sterile tube and add 100 µL chloroform and 5 mL phage buffer

Propagating T5-phages

Step 20.

Shake for 1 min to let chloroform settle out

Propagating T5-phages

Step 21.

Transfer 4 mL of the supernatant to a fresh tube

AMOUNT

4 ml Additional info:

Propagating T5-phages

Step 22.

Filter the solution with 0.22 µm pore size syringe filter to get rid of all the cellular debris

Propagating T5-phages

Step 23.

Store phage at 4ºC. They will stay infective for >1 yr.

PROTOCOL

. Fluorescent Staining of T5-phages

CONTACT: Steven Wilhelm

Preparing T5 for Staining

Step 23.1.

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 23.2.

Discard the supernatant

Preparing T5 for Staining

Step 23.3.

Resuspend the pellet with phage buffer

AMOUNT

800 µl Additional info:

Preparing T5 for Staining

Step 23.4.

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 23.5.

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 23.6.

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 23.7.

Store at 4ºC

Staining T5 for Staining
Step 23.8.

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 23.9.

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

Staining T5 for Staining
Step 23.10.

After incubation, increase the volume to 1.4 mL

Staining T5 for Staining
Step 23.11.

Centrifuge in the Beckmann TL-100 as in step 5

Staining T5 for Staining
Step 23.12.

Discard the supernatant

Staining T5 for Staining
Step 23.13.

Suspend the viruses in Milli-Q water

AMOUNT
100 µl Additional info: