

UranyLess Staining Procedure

Materials per student

1ml phage lysate ($> 5 \times 10^9$ PFU/ml)

50ul aliquot of UranyLess (assure the material is fresh with no precipitate, check expiration date before use).

Two Copper TEM grids

100ul molecular biology grade water

Slips of Whatmans paper.

**this procedure can be done using the Pelco tab method for having the copper grids on a piece of parafilm or by holding the grids in forceps.*

Procedure for immobilized grids using Pelco tab method and can be done on the benchtop.

- 1) Centrifuge 1ml of high titer phage lysate ($> 5 \times 10^9$ PFU/ml) at 6,000 rpm for 5 minutes to remove any debris or bacterial contamination.
- 2) Carefully remove the supernatant to a fresh tube being careful not to disturb any pelleted material.
- 3) Centrifuge the supernatant at $>20,000 \times g$ at 4°C for 60 minutes.
- 4) Place the tubes on ice and carefully remove all the supernatant leaving $\sim 10\text{ul}$ in the bottom of the tube.
- 5) Add 100ul fresh phage buffer and incubate on ice for 30 minutes. Do not shake or mix.
- 6) Remove 10ul of the phage sample from the bottom of the tube and place grid 1, repeat for grid 2.
- 7) Let stand for 2 minutes if phage titer is $> 5 \times 10^9$ PFU/ml, if phage titer is lower, let stand for up to 5 minutes.

**It is critical for all the washes that the grids are never left dry. As you wick the liquid have the next sample in the pipette tip and ready to be added to the grid. Work quickly but carefully*

- 8) Remove the lysate from grid 1 by wicking the liquid off and immediately add 10ul water, repeat for grid 2
- 9) Wick off the water and immediately from grid 1 and add fresh 10ul water. Repeat for grid 2
- 10) Wick off the water from grid 1 and immediately add 10ul UranyLess. Repeat for grid 2
- 11) Let stand for 1 minute and then wick off all UranyLess from both grids. It is critical to remove all the UranyLess. The grid may still look a bit wet. Let stand on the bench or in fume hood for 15 minutes to dry and then place in grid box for storage.

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