Isolation of Radiolabeled *Poliovirus* Particles from H1 HeLa Cells

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[Abstract] The following protocol describes the isolation of radioactive viral and subviral particles from infected cells. This protocol has been written for isolation of *poliovirus* particles from H1 HeLa cells. Infection protocol and timing of [^{35}S] methionine labeling and particle collection should be tailored to the virus of interest. Following isolation of the viral particles, the viral proteins present in these particles may be separated by gel electrophoresis and visualized by autoradiography.

Materials and Reagents

1. H1 HeLa cells
2. *Poliovirus*
3. Sucrose (Life Technologies, Invitrogen™, catalog number: 15503-022)
4. Methionine free DMEM (Life Technologies, Gibco®, catalog number: 21013)
5. MEM (Life Technologies, Gibco®, catalog number: 11095-080)
6. [^{35}S] Methionine (PerkinElmer, catalog number: NEG772002MC)
7. Mineral oil (Sigma-Aldrich, catalog number: M5904)
8. SDS-PAGE gel
9. Nonidet P-40 (NP-40) (Sigma-Aldrich, catalog number: N-6507)
10. Phenylmethanesulfonyl fluoride (PMSF) (Sigma--Aldrich, catalog number: P3075-1G)
11. 40 U RNAsin® Ribonuclease Inhibitor (Promega Corporation, catalog number: N2111)
12. 4x Laemelli loading buffer
13. Tris buffer (see Recipes)
14. Lysis buffer (see Recipes)
15. Gel Fixative (see Recipes)
16. Phosphate Buffered Saline (PBS) (see Recipes)
17. PBS+ (see Recipes)

Equipment

1. Centrifuge tubes (Beckman Coulter, catalog number: 344059)
2. 1.5 ml Eppendorf tubes
3. Peristaltic Pump (Bio-Rad Laboratories, model: 731-9001EDU)
4. Ultracentrifuge (Beckman Coulter, model: Optima L-90K)
5. SW41 Ti rotor (Beckman Coulter, model: 331362)
6. Fraction collection system (Beckman Coulter, catalog number: 270-331580)
7. Gradient maker (Biocomp Gradient Master, model: 107)
8. Liquid scintillation counter (Beckman Coulter, model: LS6000IC)
9. Gel dryer (Bio-Rad Laboratories, model: 583)

Procedure

A. Isolation of viral and subviral particles

1. Infect adherent cells in 10 cm dish at a MOI of 50. The virus should be diluted in 500 µl of PBS+. Incubate cells with virus alone for 30 min at 37 °C prior to the addition of 8 ml MEM. Cells should be approximately 80% confluent at the time of infection.
2. Prepare [^{35}S] methionine-DMEM by adding 100 µCi [^{35}S] methionine per milliliter of methionine free DMEM.
3. At 3 h post-infection remove media from cells, add 3 ml methionine free DMEM to cells then remove. Repeat this process for two additional washes, then add 6 ml of the [^{35}S] methionine DMEM prepared in step A-2 to cells.
4. At desired collection time points, remove [^{35}S] methionine DMEM from cells. Wash cells 3x with 3 ml PBS and add 1 ml of lysis buffer to the plate. Remove the cells from the plate by pipetting up and down several times. Then transfer the lysate (cells & lysis buffer) to a 1.5 ml tube.
5. Remove nuclei from this lysate by centrifugation at 4,500 *x g* for 10 min at 4 °C (store lysate on ice until it is added to the gradient).
6. Prepare 15% (w/v) and 30% (w/v) sucrose solutions using Tris buffer.
7. Follow gradient maker instructions for pouring a 15-30% sucrose gradient.
8. Apply 500 µl of lysate to the top of the gradient. Add approximately 200 µl of mineral oil over the radiolabeled lysate.
9. Spin tube in ultracentrifuge for 3 h at 27,500 rpm, 15 °C. The ultracentrifuge should be programmed for slow acceleration and deceleration with no brake.
10. Place tube in fraction collection system.

11. Turn on pump, to begin pumping air on top of the gradient. Immediately puncture the tube to allow sucrose to flow out of the bottom of the centrifuge tube.
12. Collect 0.5 ml fractions from the gradient into 1.5 ml tubes. The entire gradient should be collected. Fractions may be stored at -20 °C for up to one year.
13. Determine radioactivity in fractions using a liquid scintillation counter.

B. Visualization of labeled proteins in fractions
1. Mix 15 µl of each fraction with 5 µl of 4x Laemelli loading buffer.
2. Heat sample to 95 °C.
3. Separate proteins by SDS-PAGE using a 12% SDS-acrylamide gel.
4. Fix gel overnight in gel fixative at room temperature with shaking.
5. Dry gel using gel dryer.
6. Visualize proteins using standard autoradiography techniques.

Recipes

1. Tris buffer
   10 mM Tris (pH 7.4)
   10 mM NaCl
   1.5 mM $MgCl_2$
2. Lysis buffer
   10 ml Tris Buffer
   1% NP-40
   1 µM PMSF
   40 U RNAsin
3. Gel Fixative
   50% methanol
   10% acetic acid in distilled H_2O
4. PBS (makes 1 L)
   137 mM NaCl
   2.7 mM KCl
   10 mM Na_2HPO_4
   1.8 mM KH_2PO_4
   Dissolve reagents in 800 ml ddH_2O, adjust pH to 7.5, and then add ddH_2O to 1 L.
   To prepare PBS+ add 1 mM $CaCl_2 \cdot 2H_2O$ and 0.5 mM $MgCl_2 \cdot 6H_2O$.

Acknowledgments

This protocol is adapted from Richards and Jackson (2012).

References

1. Richards, A. L. and Jackson, W. T. (2012). Intracellular vesicle acidification promotes maturation of infectious *poliovirus* particles. *PLoS Pathog* 8(11): e1003046.