Purification of recombinant adenovirus

Adenoviruses are excellent vectors for gene transfer and are used extensively for high-level expression of the products of transgenes in living cells. The development of simple and rapid methods for the purification of stable infectious recombinant adenoviruses (rAds) remains a challenge. We report here a method for the purification of infectious adenovirus type 5 (Ad5) and its derivative recombinant adenovirus that involves ultracentrifugation on a cesium chloride gradient at 604,000g for 15 min at 4°C and tangential flow filtration. The entire procedure requires less than two hours and infectious viruses can be recovered at levels higher than 64 percent of the number of plaque-forming units (pfu) in the initial crude preparation of viruses. We have obtained titers of infectious purified Ad5 of 1.35×10^{10} pfu/ml and a ratio of particle titer to infectious titer of seven. The method described here allows the rapid purification of rAds for studies of gene function in vivo and in vitro, as well as the rapid purification of Ad5.

1. Propagation of virus

1. Start with 225 cm^2 flask or three 10-cm dishes.
2. Infect host cells with virus.
3. Incubate cultured cells for 2 to 3 days at 37°C.
4. Pellet infected cells.

   Tips: You may store infected cells at -80°C at this step.

2. Purification of virus

1. Suspend cells in 10 ml of sonication buffer.
2. Sonicate or freeze-and-thaw the virus suspension.
3. Centrifuge at 9,400 g for 10 min at 4°C.
4. Filtrate supernatant through 0.2-um polyethersulfone filter unit (Millipore).
5. Concentrate flowthrough into 1 ml by tangental flow filtration with Amicon Ultra-15 MWCO 100K filter (Millipore) at 5,000 g for 15 min at 4°C.
6. Spin at 604,000 g for 15 min at 4°C by ultracentrifugation on a CsCl gradient.

   Tips: Use 5 ml seal tube. Layer virus solution (ca. 1 ml), 2.2M CsCl (3 ml), and 4.0M CsCl (1 ml) in this order.
7. Recover 1 ml of virus sample.

3. Removal of CsCl

1. Add 9 ml of displacement buffer.
2. Concentrate flowthrough into 1 ml by tangental flow filtration with Amicon Ultra-15 MWCO 100K filter (Millipore) at 2,500 g for 5 min at 4°C.
3. Add 9 ml of displacement buffer directly to filtration unit.

   Tips: Suspend well before following centrifugation.
4. Concentrate flowthrough into 1 ml by tangental flow filtration with Amicon Ultra-15 MWCO 100K filter (Millipore) at 2,500 g for 5 min at 4°C.
5. Transfer virus solution to a new tube.

4. Reagent

sonication buffer (1000 ml)

10 mM HEPES, pH 8.0 (10 ml/1 M)

2.2M CsCl (100 ml)

2.2 M CsCl (38 g)

10 mM HEPES, pH 8.0 (1 ml/1 M)

4.0M CsCl (100 ml)

4.0 M CsCl (67 g)

10 mM HEPES, pH 8.0 (1 ml/1 M)

displacement buffer (1000 ml)

10 mM HEPES, pH 8.0 (10 ml/1 M)

2 mM $MgCl_2$ (10 ml/0.2 M)

10% glycerol (100 ml)

4% sucrose (40 g)

5. Reference

Ugai H, Yamasaki T, Hirose M, Inabe K, Kujime Y, Terashima M, Liu B, Tang H, Zhao M, Murata T, Kimura M, Pan J, Obata Y, Hamada H, Yokoyama KK.(2005) Purification of infectious adenovirus in two hours by ultracentrifugation and tangential flow filtration. Biochem. Biophys. Res. Commun. 331: 1053-1060.