

Adenovector™ Rapid Titer Kit

Cat. No. VSA-0024

PLEASE READ ENTIRE PROTOCOL BEFORE STARTING

The Adenovector™ Rapid Titer Kit provides a complete set of materials for the fast quantification of adenoviral stocks. The results are obtained within 48 hours and are comparable to endpoint dilution titration assay, a method that normally takes up to 2 weeks to perform. Another advantage of using this kit instead of a nucleic acid based assay is that the kit only assays for functional adenoviral particles, not including defect particles.

Kit Contents

The reagents provided are suitable for 60 titrations or 5 x 12-well plates.

- 30 µl Mouse Anti-Hexon Antibody
- 30 µl Goat Anti-Mouse Antibody (HRP conjugate)
- 1.5 ml 3% H₂O₂
- 6 ready-to-use DAB tablets
- 100 µl Adeno-X-LacZ Viral Stock as positive control.

Storage

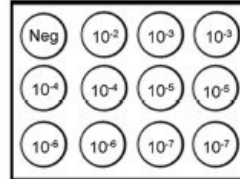
- Store 3% H₂O₂ at +4°C
- Store Antibodies and DAB tablets at -20°C
- Store Adeno-X-LacZ Viral Stock at -70°C

Procedure

Each dilution of virus should be assayed in duplicate to ensure accuracy. Assay the provided Adeno-X-LacZ Viral Stock of known titer as a positive control.

A. Infect Cells

1. Seed 293 cells (5×10^5 cells/ml) in each well of a 12-well plate.
2. Using PBS or medium as diluent, prepare 10-fold serial dilutions of your viral samples in the following way from 10^{-2} to 10^{-6} .
For example: 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} .
10 µl Adenoviral Stock diluted in 990 µl diluent = 10^{-2} ml.
3. Add 100 µl of viral dilution drop-wise to each well. Note:
Perform duplicated infections to ensure accurate assay results.
4. Incubate cells at 37°C in 5% CO₂ for 48 hours.
5. Aspirate medium.



B. Fix Cells and Add Antibodies

1. Aspirate medium and fix cells by gently adding 1 ml ice-cold 100% methanol to each well.
2. Incubate the plate at -20°C for 10 minutes.
3. Aspirate methanol. Gently rinse the wells three times with 1 ml PBS + 1% BSA.
4. Dilute Mouse Anti-Hexon Antibody 1:1,000 in PBS + 1% BSA.
5. Aspirate final rinse from wells. Then add 0.5 ml of Anti-hexon Antibody dilution to each well. Incubate for 1 hour at 37°C on an orbital shaker.
6. Aspirate Mouse Anti-Hexon Antibody. Then gently rinse wells three times with 1 ml PBS + 1% BSA.
7. Dilute Rat Anti-Mouse Antibody (HRP conjugate) 1:1,000 in PBS + 1% BSA.
8. Aspirate final rinse from wells. Then add 0.5 ml Rat Anti-Mouse Antibody (HRP conjugate) dilution to each well. Incubate 1 hour at 37°C on an orbital shaker.
9. Prior to removing the Rat Anti-Mouse Antibody (HRP conjugate), prepare DAB working solution by dissolving one DAB tablet in 10 ml of ddH₂O plus 50 µl of 3% H₂O₂. Note: To prepare DAB working solution, transfer one DAB tablet into a 15ml conical tube first, then, add 10ml ddH₂O to avoid incidentally transferring more than one DAB tablet into ddH₂O otherwise.
10. Aspirate Rat Anti-Mouse Antibody (HRP conjugate) dilution. Gently rinse each well three times with 1 ml PBS + 1% BSA.

C. Develop Color and Quantitate

1. After removing the final PBS + 1% BSA rinse, add 500 µl DAB working solution to each well. Incubate at room temperature for 10 minutes.
2. Aspirate DAB and add 1 ml PBS to each well.
3. Count a minimum of three fields of brown/black positive cells using a microscope with a 20X objective, and calculate the mean number of positive cells in each well. Note: Count dilutions with 10% or fewer positive cells. Ideal field should contain 5 to 50 positive (black/brown) cells.
4. Calculate infectious units (ifu)/ml for each well as follows:

$$\frac{(\text{Infected cells/field}) \times (\text{fields/well})}{\text{volume virus (ml)} \times (\text{dilution factor})}$$

D. Example Calculation:

- Mean positive cells/ field = 10 at 10^{-5} dilution
 - Fields/well (20X objective) = 573 fields
 - Amount dilution added = 0.1 ml
- Therefore: ifu/ml = $(10 \text{ cells/field}) \times (573 \text{ fields/well}) / (0.1 \text{ ml}) \times (10^{-5}/\text{ml})$
= 5.73×10^9 ifu/ml