Protocol-at-a-Glance

Adeno-X™ Rapid Titer Kit
Protocol-at-a-Glance

(PT3651-2)

Please read the complete *User Manual* before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users. *PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.*

A. Infect Cells	**12 well**	**24 well**
1. Seed healthy HEK 293 cells in each well of a 12-well (or 24-well) plate. Use standard growth medium (e.g., DMEM + 10% FBS + antibiotics).	1 ml 5 x 10^5 cells	0.5 ml 2.5 x 10^5 cells
2. Using PBS or medium as diluent, prepare 10-fold serial dilutions of your viral sample from 10^{-2} to 10^{-6} ml.		
3. Add viral dilution dropwise to each well. **Note:** Each dilution of virus should be assayed in duplicate to ensure accuracy.	100 µl	50 µl
4. Incubate cells at 37°C in 5% CO_2 for 48 hr.		
5. Aspirate medium. Allow cells to dry in hood for 5 min.		
B. Fix Cells and Add Antibodies		
1. Fix cells by **very gently** adding ice-cold 100% methanol to each well.	1 ml	0.5 ml
2. Incubate the plate at –20°C for 10 min.		
3. Aspirate methanol. Gently rinse wells three times with PBS + 1% BSA.	3 x 1 ml	3 x 0.5 ml
4. Dilute Mouse Anti-Hexon Antibody 1:1,000 in PBS + 1% BSA.		
5. Aspirate final rinse from the wells. Then add Anti-Hexon Antibody dilution to each well. Incubate 1 hr at 37°C.	0.5 ml	0.25 ml
6. Aspirate Anti-Hexon Antibody. Then gently rinse the wells three times with PBS + 1% BSA.	3 x 1 ml	3 x 0.5 ml
7. Dilute Rat Anti-Mouse Antibody (HRP conjugate) 1:500 in PBS + 1% BSA.		
8. Aspirate final rinse from the wells. Then add diluted Rat Anti-Mouse Antibody (HRP conjugate) to each well. Incubate 1 hr at 37°C.	0.5 ml	0.25 ml
9. Prior to removing the Rat Anti-Mouse Antibody (HRP conjugate), prepare DAB working solution by diluting 10X DAB Substrate 1:10 with 1X Stable Peroxidase Buffer. Allow the DAB working solution to come to room temperature. **NOTE:** Do not allow 10X DAB Substrate to warm to room temperature. At times, precipitate may be observed in the 10X DAB Substrate. This precipitate does not adversely affect the performance of the kit.	(Prepare 500 µl per well)	(Prepare 250 µl per well)
10. Aspirate Rat Anti-Mouse Antibody (HRP conjugate) dilution. Gently rinse each well three times with PBS + 1% BSA.	3 x 1 ml	3 x 0.5 ml

See back of page.

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C. Develop Color and Quantitate

	12 well	24 well
1. After removing the final PBS + 1% BSA rinse, add DAB working solution to each well. Incubate at room temperature for 10 min.	500 µl	250 µl
2. Aspirate DAB and add PBS to each well.	1 ml	0.5 ml

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.
4. Calculate infectious units (ifu)/ml for each well as follows:

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

Note: See Table I below for the derivation of area counted in fields/well.

Table I. Derivation of Area Counted in Fields/Well

Objective Lenses	Eyepiece Lenses (10X)			Fields/Well		
	Total Magnification	Field Diameter	Field Area (mm^2)	12-Well Plate area = 3.8 cm^2	24-Well Plate area=2.0 cm^2	96-Well Plate area=0.32 cm^2
4X	40X	5 mm	19.6	19	10	1.6
5X	50X	4 mm	12.5	30	16	2.6
10X	100X	1.8 mm	2.54	150	79	12.6
20X	200X	0.9 mm	0.64	594	313	50

This document has been reviewed and approved by the Clontech Quality Assurance Department.