Version 24-06/24

User's Manual

Retrovirus Quantitation Kit

REF **DEIA-NS2405-6**

Σ **20T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Retroviral Quantitation Kit provides an efficient system for rapid quantitation of retrovirus titer for both viral supernatant and purified virus.

General Description

Retroviral gene transfer is a technique for efficiently introducing stable, heritable genetic material into the genome of any dividing cell type. Replication-incompetent retrovirus is usually produced through transfection of the retroviral vector into a packaging cell line. Retroviruses are classified according to the receptors used to enter host cells. Ecotropic virus can recognize a receptor found on only mouse and rat cells. Amphotropic virus recognizes a receptor found on a broad range of mammalian cell types.

The murine leukemia virus (MMLV)-based vector is the most widely used retroviral vector in gene therapy due to its ability to stably integrate its transgene into host chromosomal DNA with low immunogenicity. The titration method to determine viral titer, which is performed by overlaying viral supernatant onto target cells (e.g., NIH 3T3 cells) after serial dilution, is widely used to represent infectious viral concentration as number of colonies per volume (i.e., CFU per milliliter). However, this colony-forming assay is time consuming (7 days or more). In addition, the titers determined by different groups can vary due to inconsistent conditions used for the same titration method such as target cell type, target cell number, polycation (e.g., Polybrene) concentration, incubation temperature, and exposure time for transduction.

The Retroviral Quantitation Kit does not involve cell infection; instead it specifically measures the viral nucleic acid content of purified viruses or unpurified viral supernatant sample. In the case of unpurified viral supernatant, the kit is especially useful for determining the supernatant titer before the transduction step. The kit has detection sensitivity limit of 1.5 X 10^9 Viral Particles (VP)/mL, which is sufficient for mid or high-titer retrovirus sample. The entire procedure takes about 60 minutes. Each kit provides sufficient reagents to perform up 20 tests.

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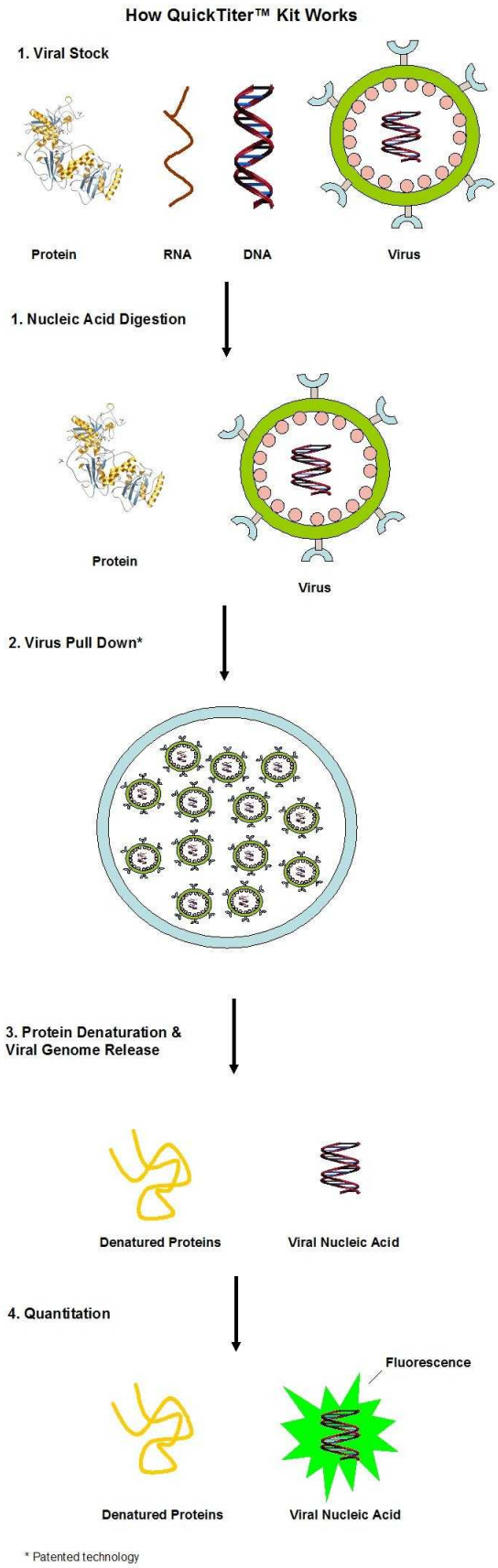
Principles of Testing


How QuickTiter™ Kit Works
1. Viral Stock
Protein
RNA
DNA
Virus
1. Nucleic Acid Digestion
Protein
Virus
2. Virus Pull Down*
3. Protein Denaturation & Viral Genome Release
Denatured Proteins
Viral Nucleic Acid
4. Quantitation
Fluorescence
Denatured Proteins
Viral Nucleic Acid
* Patented technology

Reagents And Materials Provided

1. Solution A: One tube, 200 µL.
2. Retrovirus Solution B1: One tube, 800 µL.
3. Retrovirus Solution B2: One tube, 800 µL.
4. Solution C (2×): Two tubes, 1.5 mL each
5. CyQuant GR Dye (400×): One tube, 50 µL.
6. Retrovirus RNA Standard: One tube, 500 µL containing 200 µg/mL retrovirus RNA Standard

Materials Required But Not Supplied

1. Retrovirus Sample: purified virus or unpurified viral supernatant
2. Cell Culture Centrifuge
3. 0.45 µm filter
4. 1× TE (10 mM Tris, pH 7.5, 1 mM EDTA)
5. Fluorescence Plate Reader

Storage

Store all kit components at 4°C

Reagent Preparation

1. 1× Solution C: Prepare a 1× Solution C by diluting the provided 2× stock 1:2 in deionized water. Store the diluted solution at room temperature.
2. 1× CyQuant GR Dye: Estimate the amount of 1× CyQuant GR Dye needed based on the number of assays including retrovirus RNA standard samples. Immediately before use, prepare a 1× CyQuant GR Dye by diluting the provided 400× stock 1:400 in 1× TE. For best results, the diluted solution should be used with 2 hrs of its preparation.

Preparation of Standard Curve

1. To create retrovirus RNA standards from 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL,… 0 µg/mL (1:2 serial dilution), label nine microcentrifuge tubes #1 to #9.
2. Add 20 µL of 1× Solution C to tube #2 to #9, transfer 20 µL of 200 µg/mL Retrovirus RNA Standard to tube #1 and #2. Mix tube #2 well, transfer 20 µL of the mixture (100 µg/mL) to the next tube. Repeat the steps through tube #8 and use tube #9 as a blank.
3. Transfer 5 µL of each dilution including blank to a microtiter plate suitable for fluorometer. Add 95 µL of 1× CyQuant GR Dye to each of the wells containing the 5 µL sample. Read the plate with a fluorescence plate reader using a 480/520 nm filter set.

Assay Procedure

1. Produce retrovirus in packaging cell lines with desired methods.
2. Add viral sample (up to 2 mL) to a microcentrifuge tube and adjust the final volume to 2 mL with complete culture medium such as DMEM containing 10% FBS. **Note: A proper negative control MUST be included for accurate quantitation. Use the same volume of untransfected or mock transfected packaging cell culture medium supernatant.**
3. Add 10 µl of Solution A to the assay tube and mix by inverting the tube several times. Incubate at 37°C for 30 minutes.
4. Add 20 µL of Retrovirus Solution B1, mix by inverting. Immediately add 20 µL of Retrovirus Solution B2 and mix by inverting. Incubate at 37°C for 30 minutes.
5. Centrifuge for 10 minutes at 12,000 g. Carefully remove and discard supernatant. To remove the last bit of liquid, centrifuge the tube again at 12,000 g for 30 seconds, and remove remaining supernatant with a small bore pipette tip to avoid aspirating the pellet.
6. Add 20 µL of 1× Solution C to dissolve the pellet, mix well by vortexing for 10 seconds.
7. Centrifuge 5 minutes at 12,000 g. Transfer 5 µL supernatant to a microtiter plate suitable for fluorometer. Add 95 µL of freshly prepared 1× CyQuant GR Dye to well(s) containing the 5 µL supernatant. Read the plate with a fluorescence plate reader using a 480/520 nm filter set.
8. Calculate retrovirus virus titer based on the standard curve.

Calculation

Calculation of Retrovirus Titer (VP/mL)

1. Determine Viral RNA amount:

1) Calculate Net RFU (Relative Fluorescence Unit):

Net RFU = RFU (viral sample) – RFU (negative control corresponding to viral sample)

2) Use the standard curve to determine the viral RNA amount of each unknown sample.

2. Calculate Viral Titer:

The average genome size of recombinant MMLV is 8 kb, therefore,

$$1 \text{ ng MMLV retroviral RNA} = (1\times10^{-9}) \text{ g} / (8{,}000 \text{ bases} \times 330 \text{ g/base}) \times 6 \times 10^{23} = 2.3 \times 10^{8} \text{ VP}$$

$$\text{Virus Titer (VP/mL)} = \frac{\text{Amount of retroviral RNA (ng)} \times 2.3 \times 10^{8} \text{ VP} \times (20\ \mu\text{L}/5\ \mu\text{L})}{\text{Viral sample volume (mL)}}$$

$$\text{Virus Titer (VP/mL)} = \frac{\text{Amount of retroviral RNA (ng)} \times 9.1 \times 10^{8} \text{ VP/ng}}{\text{Viral sample volume (mL)}}$$

Examples of VSVG pseudotyped GFP Retrovirus Titer Quantitation:

Method: MMLV packaging cells were cotransfected with GFP retroviral expression construct and VSVG plasmid. Medium containing VSVG pseudotyped retrovirus was harvested and filtered after 48 hrs. Retrovirus was concentrated 10 fold by centrifugation (50,000 g for 90 minutes). The concentrated viral supernatant titer was determined as described in assay instructions.

Concentrated Retroviral Supernatant: 1.0 mL was used

Average Net RFU = 39 RFU or 70 ng of viral RNA

$$\text{Virus Titer (VP/mL)} = \frac{70 \text{ (ng)} \times 9.1 \times 10^{8} \text{ VP/ng}}{1.0 \text{ mL}} = 6.4 \times 10^{10} \text{ VP/mL}$$

Note: The calculated result is the retrovirus physical titer, and it is NOT the infectious titer (TU/mL). When the infectious titer is determined, the results vary among different target cell lines or transduction methods. For reasonably packaged retrovirus vector, 1 TU is about 100 to 1000 VP.

Typical Standard Curve

The following figures demonstrate typical quantitation results. One should use the data below for reference only. This data should not be used to interpret actual results.

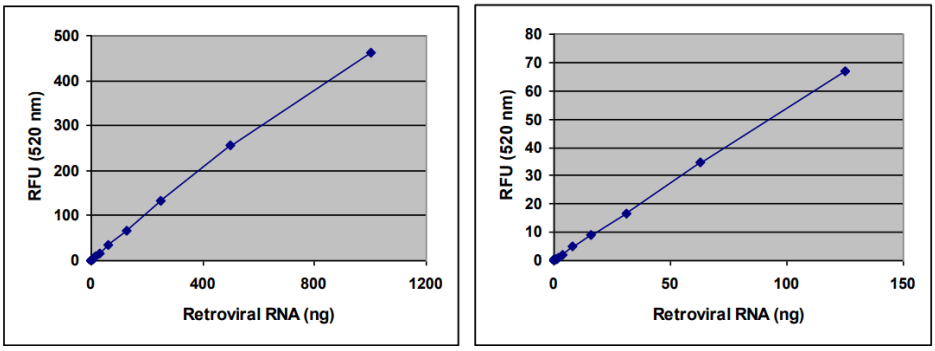


Figure 1: Retrovirus RNA Standard Curve. The Retrovirus RNA Standard was diluted as described in the above instructions. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff.

Precautions

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms.