Product Manual

QuickTiter™ Retrovirus Quantitation Kit

Catalog Number

VPK- 120 **20 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

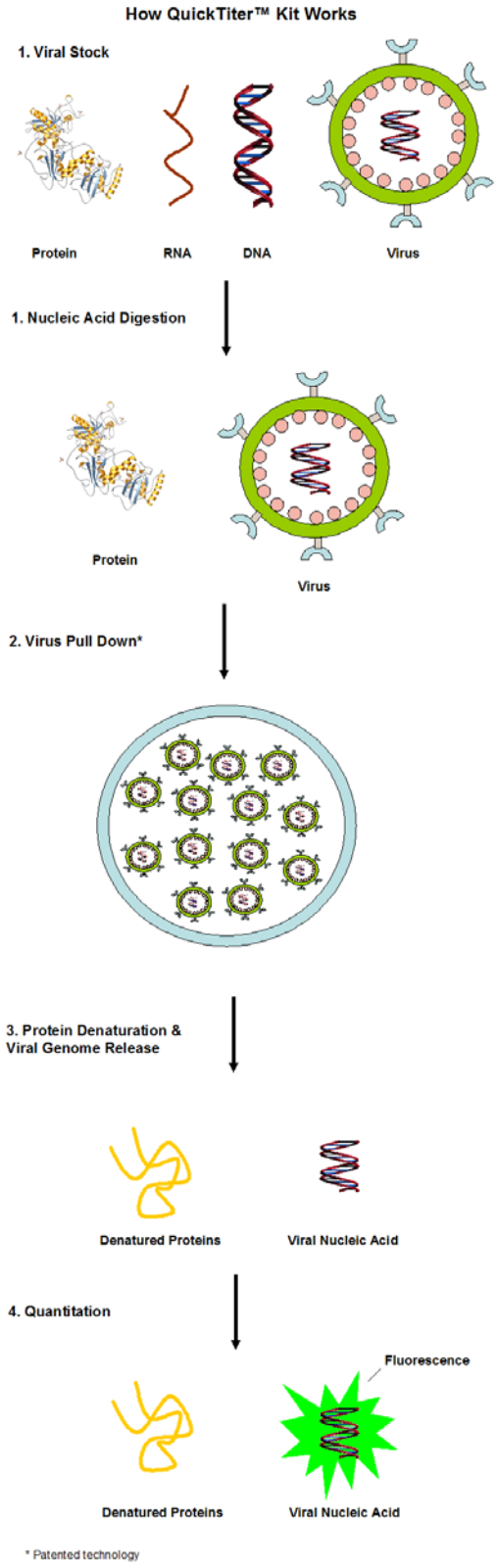
Retroviral gene transfer is a technique for efficiently introducing stable, heritable genetic material into the genome of any dividing cell type. Replication-incompetent retrovirus is usually produced through transfection of the retroviral vector into a packaging cell line. Retroviruses are classified 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.

Cell Biolabs' proprietary QuickTiter™ 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 (See Test Principle). 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 1.5×10^9 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.

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

Assay Principle



Related Products

1. RV-101: Platinum-E Retroviral Packaging Cell Line, Ecotropic
2. RV-200: ViraDuctin™ Retrovirus Transduction Kit
3. VPK-106: QuickTiter™ Adenovirus Quantitation Kit
4. VPK-109: QuickTiter™ Adenovirus Titer Immunoassay Kit
5. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
6. VPK-112: QuickTiter™ Lentivirus Quantitation Kit
7. VPK-145: QuickTiter™ AAV Quantitation Kit

Kit Components

1. QuickTiter™ Solution A (Part No. 90020): One tube – 200 µL.
2. QuickTiter™ Retrovirus Solution B1 (Part No. 312001): One tube – 800 µL.
3. QuickTiter™ Retrovirus Solution B2 (Part No. 312002): One tube – 800 µL.
4. QuickTiter™ Solution C (2X) (Part No. 90023): Two tubes – 1.5 mL each
5. CyQuant® GR Dye (400X) (Part No. 105101): One tube – 50 µL.
6. QuickTiter™ Retrovirus RNA Standard (Part No. 312003): One tube – 500 µL containing 200 µg/mL retrovirus RNA Standard

Materials Not Supplied

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

Storage

Store all kit components at 4ºC.

Safety Considerations

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

Preparation of Reagents

- 1X QuickTiter™ Solution C: Prepare a 1X QuickTiter™ Solution C by diluting the provided 2X stock 1:2 in deionized water. Store the diluted solution at room temperature.

- 1X CyQuant® GR Dye: Estimate the amount of 1X CyQuant® GR Dye needed based on the number of assays including retrovirus RNA standard samples. Immediately before use, prepare a 1X CyQuant® GR Dye by diluting the provided 400X stock 1:400 in 1X 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 1X QuickTiter™ Solution C to tube #2 to #9, transfer 20 µL of 200 µg/mL QuickTiter™ 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 1X 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 Protocol

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 QuickTiter™ Solution A to the assay tube and mix by inverting the tube several times. Incubate at 37ºC for 30 minutes.
4. Add 20 µL of QuickTiter™ Retrovirus Solution B1, mix by inverting. Immediately add 20 µL of QuickTiter™ Retrovirus Solution B2 and mix by inverting. Incubate at 37ºC 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 1X QuickTiter™ 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 1X 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.

Example of Results

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.

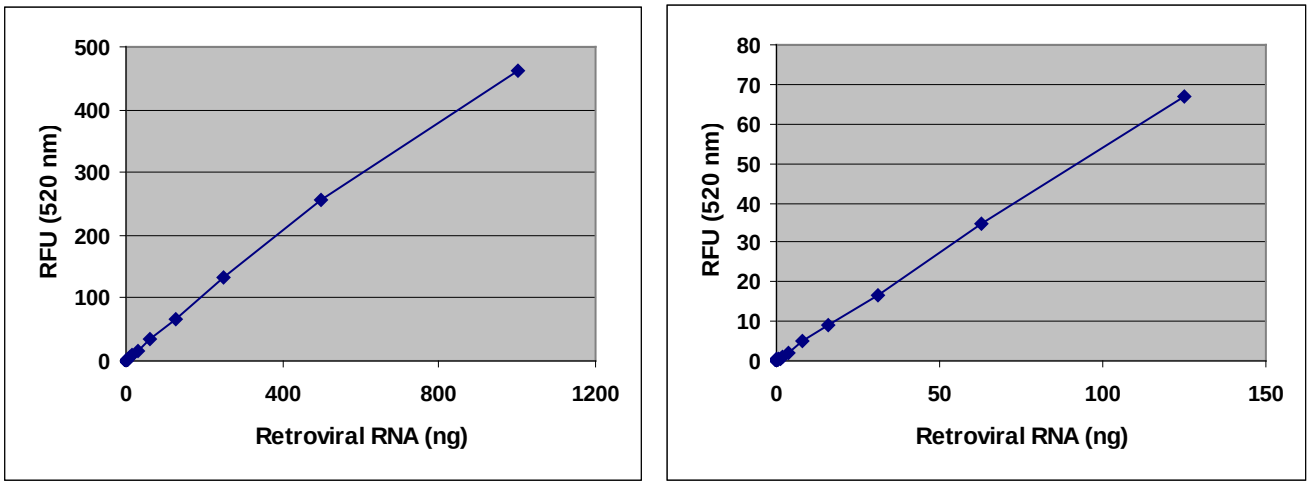


Figure 1: Retrovirus RNA Standard Curve. The QuickTiter™ Retrovirus RNA Standard was diluted as described in the above instructions. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff.

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 ng MMLV retroviral RNA = $(1x10^{-9})$ g / (8,000 bases x 330 g/base) X 6 x 10^{23} = 2.3 x 10^{8} VP

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

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

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.

Cocentrated Retroviral Supernatant: 1.0 mL was used
Average Net RFU = 39 RFU or 70 ng of viral RNA
Virus Titer (VP/mL) = $\frac{70 \text{ (ng)} \times 9.1 \times 10^8 \text{ VP/ng}}{1.0 \text{ mL}} = 6.4 \times 10^{10}$ 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[4].

References

1. Coffin, J. M. & Varmus, H. E., Ed. (1996) Retroviruses (Cold Spring Harbor Laboratory Press, NY).
2. Emi, N., Friedmann, T. & Yee, J.-K. (1991) J. Virol. 65:1202–1207.
3. Miller, A. D. & Buttimore, C. (1986) Mol. Cell. Biol. 6, 2895–2902.
4. Kwon YJ, Hung G, Anderson WF, Peng CA, and Yu H. (2003) *J Virol.* **77**, 5712-20.

Recent Product Citations

1. Zhuo, J.M. et al. (2016). Young adult born neurons enhance hippocampal dependent performance via influences on bilateral networks. *eLife* doi:10.7554/eLife.22429.
2. Rhee, Y. H. et al. (2016). Neural stem cells secrete factors facilitating brain regeneration upon constitutive Raf-Erk activation. *Sci Rep*. doi:10.1038/srep32025.
3. Manian, K. V. et al. (2015). Understanding the molecular basis of heterogeneity in induced pluripotent stem cells. *Cell Reprogram*. **17**:427-440.
4. Ito, T. et al. (2012). Stem Cell Factor Programs the Mast Cell Activation Phenotype. *J. Immunol.* **188**:5428-5437.

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