Product Manual

QuickTiter™ MuLV Core Antigen ELISA Kit (MuLV p30)

Catalog Numbers

VPK-156 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Murine leukemia viruses (MuLVs or MLVs) are retroviruses known for their ability to infect and cause cancer in murine hosts and other vertebrates. Moloney murine leukemia virus-based retroviral vectors are widely used for gene transfer to various target cells. Recently discovered in humans, xenotropic murine leukemia virus-related virus (XMRV) is closely related to murine leukemia viruses (e.g. Gag p30 core antigens share 96% identity). Early studies have linked XMRV to prostate cancer and chronic fatigue sydrome; however, new findings have disputed these claims.

Cell Biolabs' QuickTiter™ MuLV Core Antigen ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the MuLV core antigen protein (Gag p30). The kit has a detection sensitivity limit of 300 pg/mL MuLV p30. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and MuLV samples.

Assay Principle

An anti-MuLV p30 monoclonal coating antibody is adsorbed onto a microtiter plate. MuLV core antigen (p30) present in the sample or standard binds to the antibodies adsorbed on the plate; an anti-MuLV p30 polyclonal antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a HRP-conjugated secondary antibody is added and binds to the anti-MuLV p30 polyclonal. Unbound HRP-conjugated secondary antibody is removed during the wash steps, and substrate solution reactive with HRP is added to the wells.

A colored product is formed in proportion to the amount of MuLV core antigen present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from recombinant MuLV p30 core antigen and sample concentration is then determined.

Related Products

1. VPK-150: QuickTiter™ HBV Core Antigen ELISA Kit
2. VPK-151: QuickTiter™ HCV Core Antigen ELISA Kit
3. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
4. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV p24 ELISA)
5. VPK-112: QuickTiter™ Lentivirus Quantitation Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-MuLV p30 Antibody Coated Plate (Part No. 315601): One strip well 96-well plate.
2. Anti-MuLV p30 Polyclonal Antibody (Part No. 315602): One 20 µL vial.
3. Secondary Antibody, HRP Conjugate (Part No. 231009): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. Triton X-100 Solution (Part No. 310805): One 15 mL bottle containing 5% Triton X-100 in TBS.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.

7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Recombinant MuLV p30 Standard (Part No. 315603): One 100 µL vial of 10 µg/mL recombinant, MuLV Core Antigen in PBS, 500 mM Imidazole, 0.1% BSA, 0.01% NaN_3.

Materials Not Supplied

1. MuLV Sample: purified virus or unpurified viral supernatant
2. Cell Culture Centrifuge
3. 0.45 µm filter
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Recombinant MuLV p30 Standard at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

Safety Considerations

Remember that your samples contain infectious viruses before inactivation; you must follow the recommended NIH guidelines for all materials containing infectious organisms.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-MuLV p30 Polyclonal Antibody and Secondary Antibody, HRP Conjugate: Immediately before use dilute the Anti-MuLV p30 Polyclonal Antibody 1:1000 and Secondary Antibody, HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of Recombinant MuLV p30 Standard in the concentration range of 20 ng/mL – 0.313 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	MuLV p30 Standard (µL)	Assay Diluent (µL)	MuLV p30 Concentration
1	2 of Stock Standard (10 µg/mL)	998	20 ng/mL
2	300 of Tube #1	300	10 ng/mL
3	300 of Tube #2	300	5 ng/mL
4	300 of Tube #3	300	2.5 ng/mL
5	300 of Tube #4	300	1.25 ng/mL
6	300 of Tube #5	300	0.625 ng/mL
7	300 of Tube #6	300	0.313 ng/mL
8	0	300	0

Table 1. Preparation of MuLV p30 Standard

2. Transfer 225µL of each dilution (Standard Tubes 1-8) to a microcentrifuge tube containing 25 µL of Triton X-100 Solution. Perform the assay as described in Assay Instructions.

MuLV Sample Dilution and Inactivation

1. (Optional) Dilute MuLV samples in culture medium or assay diluent. Include culture medium or assay diluent as a negative control.
2. Transfer 225 µL of each sample to a microcentrifuge tube containing 25 µL of Triton X-100 Solution, Vortex well.
3. Incubate 30 minutes at 37ºC.

   Note: For samples that contain anti-MuLV p30 antibody, samples should be incubated at 56ºC for 30 min.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each MuLV sample, MuLV p30 standard, blank, and control medium should be assayed in duplicate.
3. Add 100 µL of inactivated sample or MuLV p30 standard to Anti-MuLV p30 Antibody Coated Plate.
4. Cover with a plate cover and incubate at room temperature for 2 hours on an orbital shaker.
5. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 µL of the diluted Anti-MuLV p30 Polyclonal Antibody to each well.
7. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
8. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 5 above.
9. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate to all wells.

10. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
11. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 5 above. Proceed immediately to the next step.
12. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
13. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
14. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

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

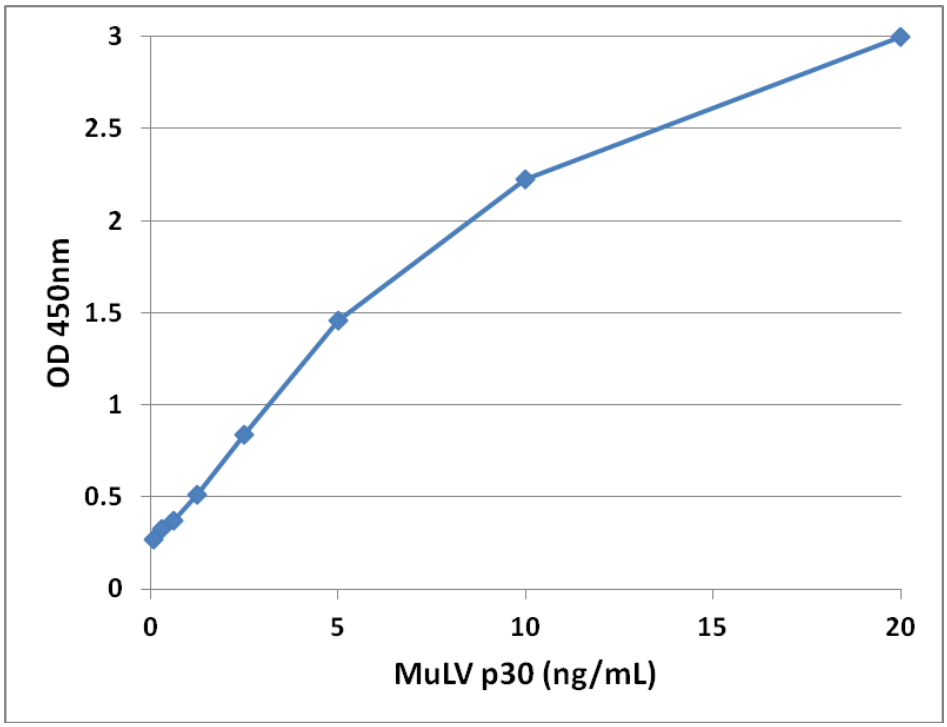

Figure 1: MuLV Core Antigen ELISA Standard Curve

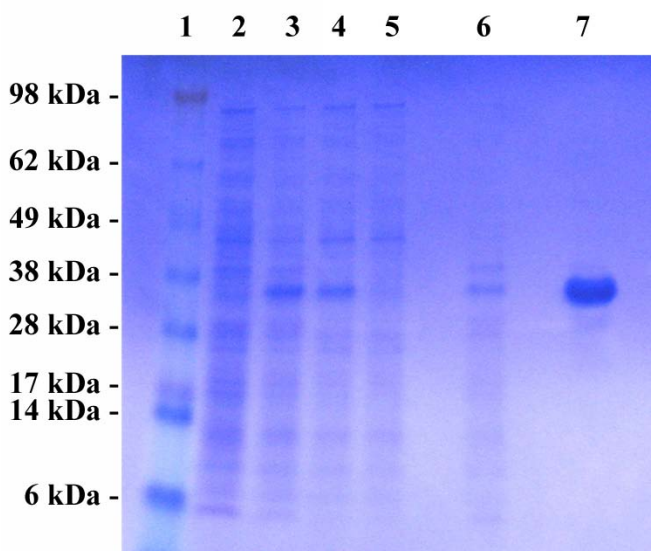

Figure 2: Purification of Recombinant MuLV p30 Protein. Lane 1: MW STDs; Lane 2: E.Coli Whole Lysate (-IPTG); Lane 3: E.Coli Whole Lysate (+IPTG); Lane 4: Crude Lysate; Lane 5: Lysate after Ni-NTA beads; Lane 6: Bead Wash; Lane 7: Elution Fraction for Recombinant MuLV p30 Standard.

Appendix

Recombinant p30 Sequence: MuLV p30 is underlined.

MASMTGGQQMGRGSPLRAGGNGQLQYWPFSSSDLYNWKNNNPSFSEDPGKLTALIESVLITH
QPTWDDCQQLLGTLLTGEEKQRVLLEARKAVRGDDGRPTQLPNEVDAAFPLERPDWDYTTQ
AGRNHLVHYRQLLLAGLQNAGRSPTNLAKVKGITQGPNESPSAFLERLKEAYRRYTPYDPED
PGQETNVSMSFIWQSAPDIGRKLERLEDLKNKTLGDLVREAEKIFNKRETPEEREERIRRETEE
KEERRRTEDEQKEKERDRRRHREMSKLLEHHHHHH-

References

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3. D'Souza V, Summers MF (2004) Nature 431: 586–90.
4. Urisman A, Molinaro RJ, Fischer N, et al. (2006) PLoS Pathogens 2: e25.

Recent Product Citations

1. Rosales Gerpe, M. C. et al. (2015). N-linked glycosylation protects gammaretroviruses against deamination by APOBEC3 proteins. *J Virol*. **89**:2342-2357.
2. Aydin, H. et al. (2014). Crystal structures of beta-and gammaretrovirus fusion proteins reveal a role for electrostatic stapling in viral entry. *J Virol*. **88**:143-153.

3. Nityanandam, R. & Serra-Moreno, R. (2014). BCA2/Rabring7 targets HIV-1 Gag for lysosomal degradation in a tetherin-independent manner. *PLoS Pathog*. **10**:e1004151.
4. Kirchmeier, M. et al. (2014). Enveloped virus-like particle expression of human cytomegalovirus glycoprotein B antigen induces antibodies with potent and broad neutralizing activity. *Clin. Vaccine Immunol*. **21**:174-180.
5. Belanger, K. et al. (2013). Binding of RNA by APOBEC3G controls deamination-independent restriction of retroviruses. *J. Exp. Biol*. **216**:2213-2220.

Warranty

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