MuLV Core Antigen / MuLV p30 ELISA Kit

Enzyme Immunoassay for the quantification of MuLV core antigen (MuLV p30) in purified virus or unpurified viral supernatant.

Catalog number: ARG82237

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

The murine leukemia viruses (MLVs or MuLVs) are retroviruses named for their ability to cause cancer in murine (mouse) hosts. Some MLVs may infect other vertebrates. MLVs include both exogenous and endogenous viruses. Replicating MLVs have a positive sense, single-stranded RNA (ssRNA) genome that replicates through a DNA intermediate via the process of reverse transcription. [Wikipedia: Murine leukemia virus]

MuLV Gag polyprotein plays a role in budding and is processed by the viral protease during virion maturation outside the cell. During budding, it recruits, in a PPXY-dependent or independent manner, Nedd4-like ubiquitin ligases that conjugate ubiquitin molecules to Gag, or to Gag binding host factors. Interaction with HECT ubiquitin ligases probably links the viral protein to the host ESCRT pathway and facilitates release.

Capsid protein p30 forms the spherical core of the virion that encapsulates the genomic RNA-nucleocapsid complex. [Uniprot]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique developed for detection and quantitation of the MuLV core Antigen protein (MuLV p30). A monoclonal antibody specific for MuLV core antigen (MuLV p30) has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any MuLV core antigen present is bound by the immobilized antibody. After washing away any unbound substances, a MuLV core antigen (MuLV p30) antibody is added to each well and incubate. Following a washing

to remove unbound substances, a HRP-conjugated secondary antibody is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of MuLV core antigen bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of MuLV core antigen in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, aliquot and store Standard at -20°C and store all other kit components at 4°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
antibody-coated microplate	12 strips X 8 wells	4°C
Standard (10 µg/ml)	100 µl	-20°C
10X Wash Buffer	100 ml	4°C
1000X MuLV core antigen Antibody concentrate	20 µl	4°C
1000X HRP-conjugated Secondary Antibody concentrate	20 µl	4°C
Triton X-100 Solution (5%)	15 ml (Ready-to-use)	4°C
Assay Diluent	50 ml (Ready-to-use)	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620 nm as reference wavelength)
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water.
- Microplate shaker.
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, aliquot and store Standard at -20°C and store all other kit components at 4°C at all times.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

1. (Optional) Dilute MuLV sample 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 to mix well.

3. Incubate the tube for 30 minutes at 37°C.

Note: For samples that contain anti-MuLV core antigen (MuLV p30) antibody, to release MuLV core antigen from the virion and to inactivate antibody. After adding Triton X-100 Solution the samples should be incubated at 56°C for 30 min instead of 37°C incubation before assay to inactivate anti-MuLV p30 antibodies.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer, mix well. Storage at 2-8°C.
- 1X MuLV core antigen Antibody working solution: Dilute the antibody immediately before use; dilute the 1000X MuLV core antigen Antibody concentrate into Assay Diluent to yield 1X MuLV core antigen antibody working solution. (E.g.: 10 µl of the MuLV core antigen Antibody concentrate (1000X) + 9.990 ml of Assay Diluent.) Do not store diluted solutions.

- **1X HRP-conjugated Secondary Antibody working solution:** Dilute the antibody immediately before use; dilute the **1000X** HRP-conjugated Secondary Antibody concentrate into **Assay Diluent** to yield 1X HRP-conjugated Secondary Antibody working solution. (E.g.: 10 µl of the HRP-Secondary Antibody concentrate (1000X) + 9.990 ml of Assay Diluent.) Do not store diluted solutions.
- **Human MuLV core antigen standard:**

1. Prepare a series dilution of recombinant MuLV core antigen (p30) standards with **Assay Diluent.** The Assay Diluent serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with Assay Diluent as according to the suggested concentration table below:

Standard No	MuLV core antigen (p30) (ng/ml)	Assay Diluent (µl)	Standards (µl)
S1	20	998	2 (10 µg/ml stock)
S2	10	500	500 (S1)
S3	5	500	500 (S2)
S4	2.5	500	500 (S3)
S5	1.25	500	500 (S4)
S6	0.625	500	500 (S5)
S7	0.313	500	500 (S6)
S0	0	500	0

2. Transfer 225µL of each dilution to a microcentrifuge tube containing 25 µl of Triton X-100 Solution. Perform the assay as described in Assay Instructions.

Note: Upon receipt, the Standard should be aliquoted and stored at ≤ -20°C to avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (18- 25°C) before use, each vial should be mixed thoroughly without foaming prior to use. Warm Substrate Solution to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the bag containing the desiccant pack, and reseal it.
2. Add **100 µl of inactivated sample or MuLV p30 standard** into the appropriate wells in the antibody coated plate. Cover the plate and incubate for **2 hour at RT** on an orbital shaker.
3. Aspirate each well and wash, repeating the process 4 times for a total **5 washes**. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µl** of the **1X MuLV core antigen Antibody working solution** to each well, cover the plate and incubate for **1 hour at RT** on a microplate shaker.
5. Aspirate each well and **wash** as step 3.
6. Add **100 µl** of the **1X HRP-conjugated Secondary Antibody working solution** to all wells, cover the plate and incubate for **1 hour at RT** on a microplate shaker.
7. **Warm TMB substrate solution to RT** before next wash step. Aspirate each well and **wash** as step 3. Proceed immediately to the next step.
8. Add **100 µl** of **TMB substrate solution** into each well. Incubate for **5-30 mins at RT** on microplate shaker. Avoid exposure to light.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Add **100 µl** of **Stop Solution** to each well and shake lightly to ensure homogeneous mixing.
10. Read the OD with a microplate reader at **450nm** immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

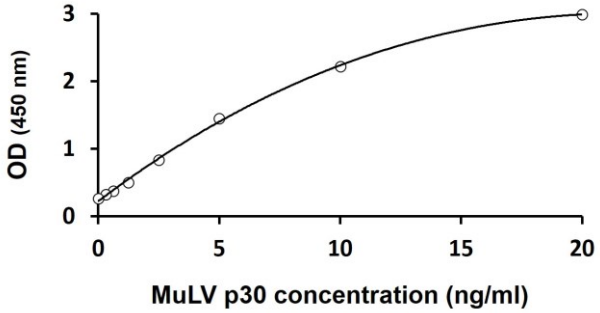
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

0.3 ng/ml

Assay Range

0.313- 20 ng/ml