Promega

Product Application

Quantify p24 Protein in Lentivirus

Quantify p24 protein in lentivirus samples using the Lumit™ p24 Immunoassay and the GloMax® Discover Microplate Reader.

Kit: Lumit™ p24 Immunoassay (Cat.# CS2039B25)

Analyses: Luminescence

Sample Type: Lentivirus

Input: 10µl

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, contact Technical Services at: **techserv@promega.com**

Materials Required:

- Lumit™ p24 Immunoassay (Cat.# CS2039B25)
- GloMax® (Cat.# GM3000)
- White, round bottom plates (e.g. Thermo Fisher Scientific Cat.# 267350)
  Note: Reduced assay performance may be observed with flat bottom, tissue culture plates
- Phosphate Buffered Saline (PBS)

Protocol:

Prepare p24 Standards:

1. Prepare an 8-point standard curve according to the following:

Tube	Viral Lysis Buffer (µl)	Volume (µl) and tube# of p24 standard	p24 concentration (pg/ml)
1	97.6	2.4 of p24 standard	60,000
2	40	40 of #1	30,000
3	40	40 of #2	15,000
4	40	40 of #3	7,500
5	40	40 of #4	3,750
6	40	40 of #5	1,875
7	40	40 of #6	937.5
8	40	40 of #7	468.8

Prepare Samples:

1. Lyse lentivirus samples by combing equal volumes of Viral Lysis Buffer and sample (e.g. add 10µl Viral Lysis Buffer to 10µl of sample).
2. After lysis, dilute the lysed samples in Viral Lysis Buffer. Subsequently, perform 2-3 dilutions of the sample to ensure that the luminescence signal falls within the dynamic range of the assay.
   a. Note: Dilution factor required will depend on the starting concentration of the lentivirus sample.

Prepare Reagents:

- Lumit™ Immunoassay Buffer A
    a. Add 2ml of Lumit™ Immunoassay Buffer A, 10X to 18ml PBS in a 50ml conical tube.
    b. Mix well.
- Anti-p24 Master Mix:
    a. Add 3mL of 1X Lumit™ Immunoassay Buffer A to a tube (e.g., a 15ml conical tube).
    b. Add 5.3µl of anti p24-SmBiT to the tube in Step a.
    c. Add 18µl of anti p24-LgBiT to the tube in Step a.
    d. Mix thoroughly by gentle inversion.

    Note: Prepare new Master Mix, as described above, immediately prior to each assay run. Do not store the Master Mix. Centrifuge the LgBiT and SmBiT tubes briefly before use to collect contents at the bottom. Do not store master mix. For running a partial plate, adjust the volume of the Master Mix accordingly and store remaining components at -20 °C.

Lumit™ p24 Immunoassay:

1. Add 20µl of anti-p24 Master Mix per well to a 96-well white, round bottom plate.
2. Add 10µl of diluted sample or p24 standard samples to the plate.
3. Cover the plate and incubate for 60 minutes with gentle shaking at room temperature.
4. Prepare Lumit™ Detection Reagent A by adding 40µl of Lumit™ Detection Substrate A to 2ml of 1X Lumit™ Immunoassay Buffer A.
5. Add 10µl Lumit™ Detection Reagent A per well and incubate for 3 minutes.
6. Read luminescence using the GloMax® Discover Microplate Reader or another luminometer.

Results:

The Lumit™ p24 Immunoassay can be used to quantify p24 protein in lentivirus samples.

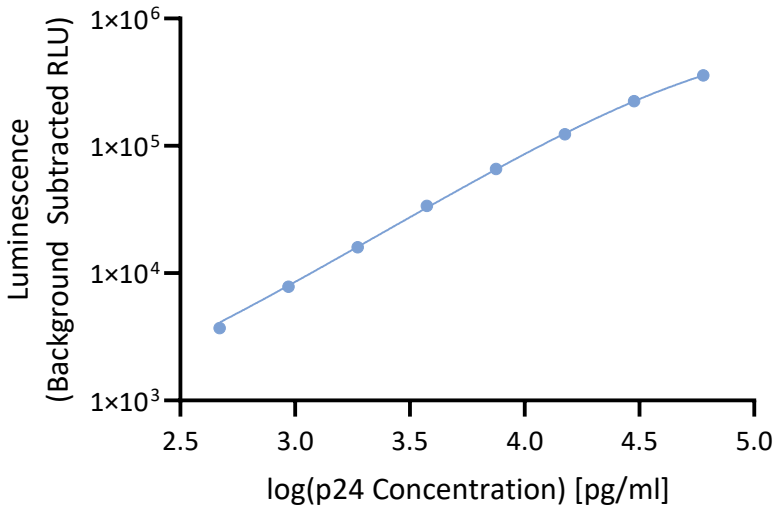

Figure 1. Lumit™ p24 Immunoassay Standard Curve. An 8-point standard curve was prepared in duplicate according to the protocol described above and fit with a 4-parameter logistic model.

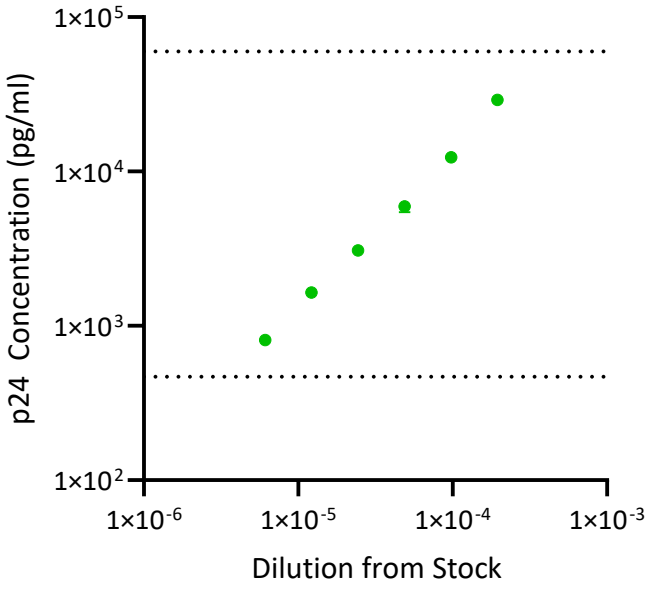

Figure 2. Concentration of lentivirus samples measured using the Lumit™ p24 Immunoassay. 2-fold serial dilutions of a lentivirus sample were prepared in Viral Lysis Buffer. p24 concentration of each sample was measured using the Lumit™ p24 Immunoassay according to the method described above. Average concentration of N=4 samples and standard deviation are shown. X-axis represents the dilution factor of the lentivirus from the stock sample. Dotted lines represent the upper and lower limits of the standard curve prepared in Figure 1.