RES50-EN.01

resDetect™ HIV-1 p24 ELISA Kit (Residue Testing)

(Enzyme-Linked Immunosorbent Assay)

Catalog Number：RES-A050

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure

INTENDED USE

The HIV-1 p24 ELISA Kit (Residue Testing) is developed for quantitative detection of Human Immunodeficiency Virus-1 p24 (HIV-1 p24) in samples from CAR-T product preparation processing where it is used as a lentivirus vector. It is intended for research use only (RUO).

BACKGROUND

Human Immunodeficiency Virus (HIV) can be divided into two major types, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). HIV-1 is related to viruses found in chimpanzees and gorillas living in western Africa. The gag gene of HIV-1 encodes a precursor protein known as Pr55Gag. The viral protease PR cleaves this precursor to generate p17, p24, p7, and p6 proteins which are required for virus particle assembly. p24 is a major viral core structural protein, which is the main component of HIV virus and plays an important role in the process of virus reproduction. Usually, p24 protein can be detected in blood 2-3 weeks after HIV infection. Its measurement is commonly used as an indicator of HIV-1 infection and viral load. Therefore, it's helpful to develop the Human Immunodeficiency Virus type 1 (HIV-1) p24 ELISA Kit to quantitative detection the HIV-1 p24 antigen in cell culture fluid.

To support the development of CAR-T drugs, ACROBiosystems independently developed HIV-1 p24 ELISA Residue Testing kit via rigorous methodological validation, which is used for detection of GMP Capsid protein p24 in samples from CAR-T product preparation processing for evaluation the quality of CAR-T products in drug development and CMC quality control stages.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of HIV-1 p24 by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-HIV-1 p24 Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-HIV-1 p24 Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The

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reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of HIV-1 p24 bound.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatant, serum and plasma samples.
3. Do not use reagents past their expiration date.
4. Do not mix or substitute reagents with those from other kits or other lot number kits.
5. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.
6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.
7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RES050-C01	Pre-coated Anti-HIV-1 p24 Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RES050-C02	HIV-1 p24 Standard	20 μg	Power	2-8°C	-70°C
RES050-C03	Biotin-Anti-HIV-1 p24 Antibody	20 μg	Power	2-8°C	-70°C
RES050-C04	Streptavidin-HRP	50 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES050-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES050-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES050-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light

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RES050-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃
RES050-C09	1×Lysis Buffer	12 mL	Liquid	2-8℃	2-8℃

SRORAGE

1. Unopened kit should be stored at 2°C -8°C upon receiving.
2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

Note: *a. Do not use reagents past their expiration date.*

b. Find the expiration date on the outside packaging.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or multi-channel micropipettes and pipette tips: need to meet 10 µL, 300 µL, 1000 µL injection requirements;

37ºC Incubator;

Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vertexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, the size of the aliquot should not be less than 10 µg.

Note: *Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for*

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even better accuracy.

Table 2. Preparation method

ID	Components	Size (96 T)	Storage solution concentration.	Reconstituted water Vol.
RES050-C02	HIV-1 p24 Standard	20 μg	100 μg/mL	200 μL
RES050-C03	Biotin-Anti-HIV-1 p24 Antibody	20 μg	100 μg/mL	200 μL

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti-HIV-1 p24 Antibody working fluid:

Dilute Biotin-Anti-HIV-1 p24 Antibody to 0.1 μg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

1.5 Sample lysate method

If the sample to be tested needs to be lysed, it is recommended to use 1×Lysis Buffer for processing, and the volume ratio of the sample to the lysate is 1:1, and the outside of the well is lysed. Lysis at room temperature for 30min can lyse about 3500~30000ng/mL of virus. The sample after lysis should be diluted at least 10-fold.

Note: *Customers can choose their own cracking method according to the specific situation, it is important to verify the lysate interference.*

2. Preparation of Standard curve

The concentration of the reconstituted HIV-1 p24 Calibrator (RES050-C02) is 100 μg/mL， prepare

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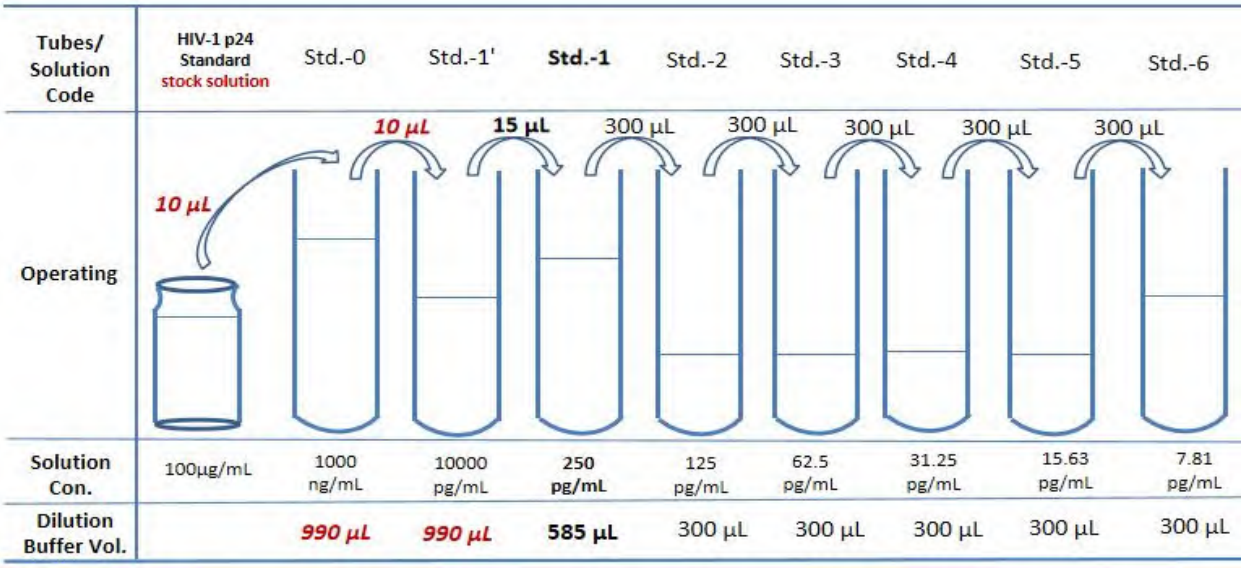
(Std.-0) by diluting 10 µL the reconstituted HIV-1 p24 Calibrator into 990 µL Sample Dilution Buffer, mix gently well. Then prepare Std.- 1' by diluting 10 µL Std.-0 into 990 µL Sample Dilution Buffer. At last, prepare the highest concentration of standard curve, Std.-1 (250 pg/mL), by diluting 15 µL Std.- 1' into 585 µL Sample Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 µL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.



3. Add Samples

Add 100 µL Calibrator and samples to each well. For blank Control wells, please add 100 µL Dilution Buffer.

***Note**: It is recommended to set double holes for samples and standard curves to be tested.*

4. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

6. Add Biotin-Anti-HIV-1 p24 Antibody

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For all wells, add 100 µL Biotin-Anti-HIV-1 p24 Antibody (dilute to 0.1 µg/mL) working solution. Please prepare it for one-time use only.

7. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

8. Washing

Repeat step 5.

9. Add Streptavidin-HRP

For all wells, add 100 µL Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

10. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

11. Washing

Repeat step 5.

12. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

13. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: *The color in the wells should change from blue to yellow.*

14. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

Note*: To reduce the background noise, subtract the value read at* OD_{450nm} *with the value read at* $OD_{630\ nm}$.

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (OD).
2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance

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value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

3. Normal range of Standard curve: $R^2 \geq 0.9900$.

4. Detection range: 7.81 pg/mL-250 pg/mL. If the OD value of the sample to be tested is higher than 250 pg/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 7.81 pg/mL, the sample should be reported.

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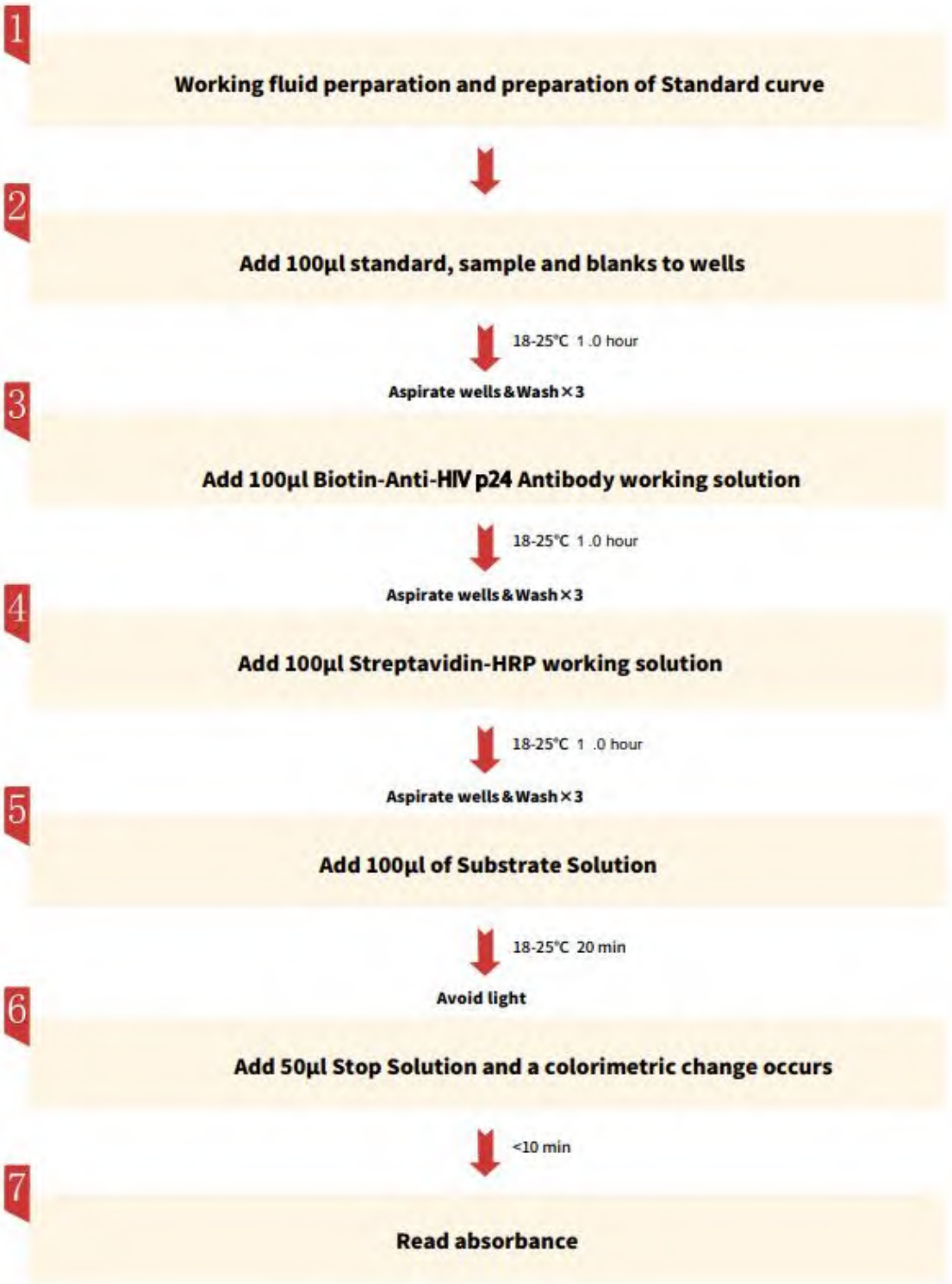
QUICK GUILD



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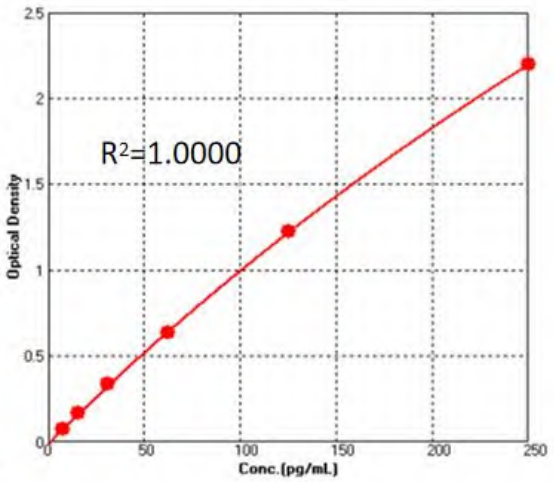
TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Conc.(pg/mL)	O.D.-1	O.D.-2	Average	Corrected
250	2.262	2.238	2.250	2.198
125	1.298	1.255	1.277	1.224
62.5	0.677	0.705	0.691	0.639
31.25	0.405	0.374	0.390	0.337
15.63	0.220	0.212	0.216	0.164
7.81	0.135	0.124	0.130	0.077
0	0.048	0.057	0.053	/



SENSITIVITY

The minimum detectable concentration of HIV-1 p24 is 1.556 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. Inter-assay Precision

Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (pg/mL)	196.596	52.265	20.803	188.580	51.137	20.538

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SD	6.866	1.940	1.370	8.630	1.707	0.308
CV (%)	3.5	3.7	6.6	4.6	3.3	1.5

***Note**: The example data is for reference only.*

RECOVERY

Five different concentrations of HIV-1 p24 were added to the cell culture supernatant, and the cell culture supernatant without HIV-1 p24 was used as the background to calculate the recovery rate.

Sample Type	Range
HEK293 cell culture supernatant (n=5)	88.6-93.0%
T cell culture supernatant (n=5)	88.0-97.1%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of HIV-1 p24 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (1640)
1:2	Average Recovery (%)	98.1	89.1
	Range (%)	89.6-110.7	84.2-93.2
1:4	Average Recovery (%)	93.9	90.8
	Range (%)	91.9-96.0	87.3-96.5
1:8	Average Recovery (%)	93.8	89.9
	Range (%)	87.9-101.9	85.9-95.0
1:16	Average Recovery (%)	97.7	95.4
	Range (%)	87.7-106.5	89.7-101.9

***Note**: The example data is for reference only.*

INTERFERING SUBSTANCES

Verify potential matrix effects by adding different levels of cell culture medium to the diluted buffer.

Additive	Tolerated concentration

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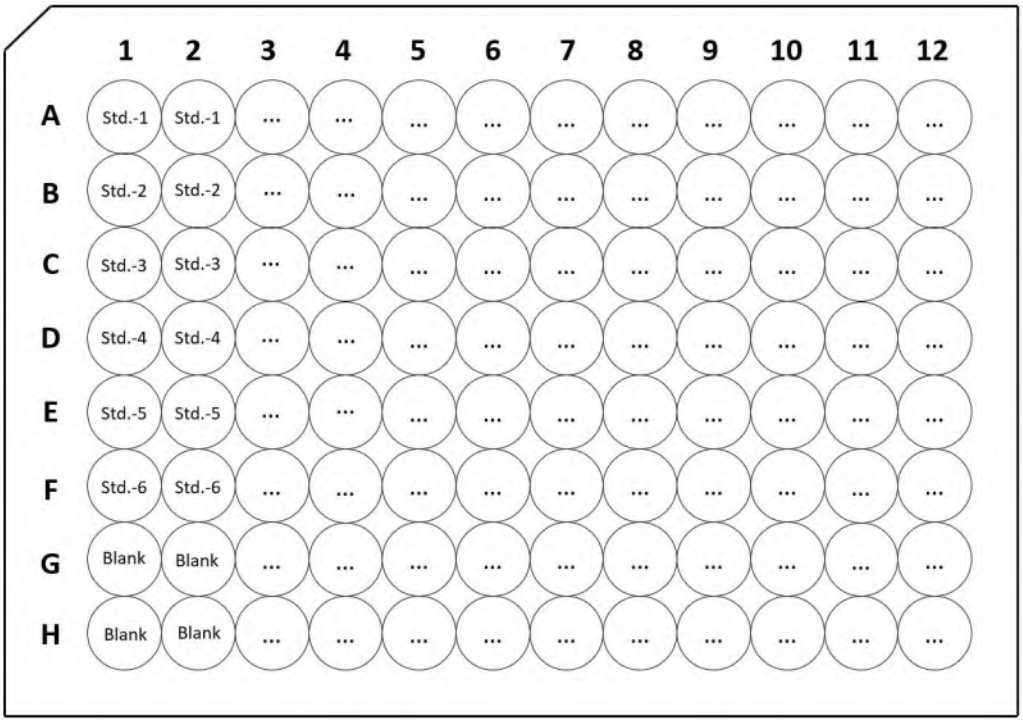
DMEM	50%
1640	50%

TITER CALCULATION

The following values and calculations may also be used to determine approximate titers, and are based on the observation that each lentiviral particle (LP) contains approximately 2000 molecules of p24:

- 1 LP contains 8×10^{-5} pg of p24 (derived from $(2000)\times(24\times10^{3}$ Da$)/(6\times10^{23})$
- 1 ng p24 is equivalent to 1.25×10^{7} LPs
- For a typical lentivirus vector, there is 1 IFU for every 100–1000 LPs
- Therefore, 1 ng p24 is equivalent to $\approx 1.25\times10^{4} \sim 1.25\times10^{5}$ TU

PLATE LAYOUT



Note: *Blank is a Blank Dilution Buffer hole.*

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TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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