Instructions for Use of Lentivirus Titer p24 ELISA Detection Kit

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG- P001L

Introduction

This product uses a double-antibody sandwich method to detect HIV-1 p24 protein in samples. A monoclonal antibody specific to HIV-1 p24 antigen is coated on a microplate, and the standard or test sample is added into the reaction well. At the same time, the anti- HIV-1 p24 secondary antibody is added and incubated at room temperature to form the antibody-antigen-secondary antibody complex. The unconjugated compounds are removed by washing and protein content in the sample is indicated by the intensity of TMB color development.

Assay range: 1.37-1,000 ng/mL

Sensitivity: 0.35 ng/mL

Precision: CV%≤10%　RE%≤±15%

Specification

96 T

Usage

This kit is applicable to rapid detection of p24 protein content in any HIV-1-based lentivirus.

Kit components

Component	Specification	Preparation
HIV-1 p24 Coated Plate	8 wells × 12 strips	Ready-to-use
Anti HIV-1 p24+Streptavidin (detection antibody conjugated	6 mL × 1 bottle	Ready-to-use
HIV-1 p24 Standard	S1-S7, S0	Ready-to-use
Virus Lysis	6 mL × 1 bottle	Ready-to-use
Sample Diluent Buffer	15 mL× 3 bottle	Ready-to-use
10×PBST Wash Buffer (10×PBST)	50 mL× 1 bottle	1:10, dilute with deionized water
Color Reagent (TMB chromogenic substrate)	12 mL× 1 bottle	Ready-to-use
Stop Solution	12 mL× 1 bottle	Ready-to-use
Plate Sealer	3 pieces	Ready-to-use
Instructions for Use (IFU)	1 copy	Ready-to-use

Note: All components should be stored at 2 - 8℃, protected from light with a shelf life of 18 months.

Apparatus and materials to be prepared by the user:

(1) Plate reader

(2) Constant temperature incubator

(3) Micro pipette and tips

(4) Deionized water

(5) Unused filter paper

(6) Vortex shaker

Reagent preparation

(1) Equilibration: Equilibrate the reagents to be used at room temperature (18 - 25℃) for 30 minutes.

(2) Preparation of solution:

1 × PBST Wash Buffer: Calculate the volume of working buffer required, measure an appropriate amount of 10 × PBST Wash Buffer, dilute with deionized water at a ratio of 1:10, and mix well for later use.

(3) Dilution of standard:

The standard provided is ready-to-use and can be directly subjected to vortexing for use after equilibration to room temperature.

Sample dilution

Dilution of crude virus samples and filtered samples (50-fold dilution): Pipette 490 μL of sample dilution into a 1.5-mL centrifuge tube, add 10 μL of crude virus sample or filtered sample, and vortex to mix well;

Chromatographic sample dilution (200-fold dilution): Pipette 995 μL of sample dilution into a 1.5-mL centrifuge tube, add 5 μL of chromatographic sample and vortex to mix well.

Operation procedures

(1) Mix all reagents well before use to avoid bubbles.

(2) Determine the number of stripe plates required based on the number of experimental wells. Put remaining strip plates back to aluminum foil bags and seal properly.

(3) Loading: Add 50 μL of lysis buffer to each well and then add 10 μL of standard, sample dilution working solution, negative control to the corresponding well; then add 50 μL of detection antibody conjugated peroxidase to each well. Seal the plate with a plate sealer and incubate on a room temperature shaker at 500 rpm for 30 minutes.

(4) Plate washing: Discard the liquid in each well, and fill the wells with 1 × PBST Wash Buffer (300 μL/well). Allow to stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 4 times, and pat the plate dry on the filter paper after each washing.

(5) Color development: Add 100 μL of TMB Substrate to each well, mix well by gentle shaking, seal the plate with a plate sealer, and place the plate at room temperature for 5-10 minutes for color development reaction.

(6) Assay: Add 100 μL of Stop Solution to each well and mix well by gentle shaking. Measure the optical density (OD) value of each well with a microplate reader at a primary wavelength of 450 nm and a reference wavelength of 630 nm.

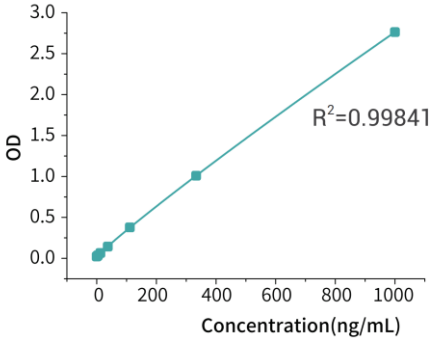
Results processing

The 4-parameter fitting method is recommended for the linear fitting and calculation using the product.

(1) OD processing of the standard curve (the following example is provided as reference only, and the results from actual detection shall prevail)

Standard concentration (ng/mL)	OD value (1)	OD value (2)	Mean value
1000	2.783	2.744	2.7635
333.33	1.029	0.988	1.0085
111.11	0.377	0.379	0.3780
37.04	0.143	0.144	0.1435
12.35	0.066	0.064	0.0650
4.12	0.035	0.036	0.0355
1.37	0.026	0.026	0.0260
0	0.022	0.022	0.0220

(2) The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below)



Precautions

(1) When the sample is tested for the first time, it is recommended to perform dilution with at least 3 consecutive dilution factors, so as to generate at least one diluted sample within the range of the standard curve.

(2) The reagents should be stored as indicated on the label, and should be equilibrated to room temperature before use.

(3) Before using the coated microtiter plates, please equilibrate to room temperature and then open the secondary packaging. The strip plates not used in the test should be immediately placed back into the package and sealed properly, and can be stored at 4℃ for one month. Other unused reagents should be packaged or covered properly.

(4) The volumes of standard, biotin, and enzyme conjugate are all very small. Please perform rapid and brief centrifugation before use to settle the liquid adherent on the tube wall or cap at tube bottom.

(5) Please use disposable tips during experimental operation to avoid cross contamination.

(6) Please check each individual reagent in the kit fully before use. To obtain accurate assay results, it is of special importance to mix well or shake well the reagents for dilution, loading, and reaction termination.

(7) When washing residual Wash Buffer in the reaction wells, pat the plate dry adequately on clean tissue papers until watermark is no longer visible. Do not put the tissue paper into the well for liquid absorption.

(8) The TMB Substrate is photosensitive, thus long-time exposure to illumination should be avoided; avoid contact with metal, otherwise, the assay results may be affected.

(9) The kit is intended for single use. Please use within the shelf life.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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