Instructions for Use of HIV-1 p24 ELISA Detection Kit

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

Cat. No. HG- P001

Introduction

This product uses a double-antibody sandwich method to detect HIV-1 p24 protein in samples, which involves pre-coating microplate wells with a monoclonal antibody specific to HIV-1 p24 antigen, and then adding the standard and test sample into the coated reaction wells for incubation. HIV-1 p24 protein present will quantitatively bind to the antibody in the microplates, and the test procedure is to remove the unbound complex by washing; add the biotin-labeled anti-p24 antibody, and finally add avidin-labeled HRP to form an antibody-antigen-biotin-enzyme-labeled avidin complex, and indicate the protein content in the sample by observing the degree of TMB color development. Please read the instructions for use carefully and check the components of the kit before use.

Assay range: 6.25-200 pg/mL
Limit of quantification: 6.25 pg/mL
Limit of detection: 3.125 pg/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

Components	Specification	Preparation
HIV-1 p24 Coated Plate	8 wells × 12 strips × 1 piece	Ready-to-use
Anti-p24-Biotin (detection antibody)	150 μL × 1 vial	1:100, and dilute in 20% CS dilution buffer
Streptavidin HRP (enzyme conjugate)	150 μL × 1 vial	1:100, and dilute in 20% CS dilution buffer
HIV-1 p24 Standard	30 μL × 1 vial (0.48 mg/mL)	Operate as per the recommended dilution procedure
Lysis Buffer	1.5 mL × 1 vial	Ready-to-use
20%CS Buffer	25 mL× 1 bottle	Ready-to-use
Sample Diluent Buffer	50 mL× 1 bottle	Ready-to-use
20×PBST Wash Buffer (20×PBST)	50 mL× 1 bottle	1:20, dilute in deionized water
Color Reagent A	7 mL× 1 bottle	Ready-to-use
Color Reagent B	7 mL× 1 bottle	Ready-to-use
Stop Solution	7 mL× 1 bottle	Ready-to-use
Plate Sealer	5 pieces	Ready-to-use
Instructions for Use	1 copy	Ready-to-use

Note: The detection antibody, enzyme conjugate, standard and 20% CS Buffer should be stored at -18℃, and other components should be stored at 2-8℃, protected from light, with a shelf life of 12 months.

Apparatus and materials to be prepared by the user:

(1) Plate reader
(2) Thermostat plate shaker
(3) Micro pipette and tips
(4) Deionized water
(5) Unused filter paper
(6) Vortex shaker

Reagent preparation

(1) Temperature equilibration: Transfer reagents to be used to room temperature (18 ~ 25℃) environment and equilibrate the temperature for 30 minutes.

(2) Preparation:

① 1 × PBST Wash Buffer: Calculate the volume of working solution required for the test, transfer an appropriate amount of 20 × PBST Wash Buffer, dilute with deionized water in a ratio of 1:20, and mix well for later use.

② Detection antibody and enzyme conjugate working buffer

: Calculate the volume of working solution required for the test, transfer an appropriate amount of biotin antibody or enzyme conjugate, dilute with 20% CS Buffer in a ratio of 1:100, and mix well for later use.

③ The standard and test samples should be diluted with the Diluent Buffer.

(3) Standard dilution: Store the first intermediate gradient (Pre-1) of the standard at -20℃ for 1-7 days.

Vial No.	Standard solution concentration (pg/mL)	Standard solution volume (μL)	Diluent Buffer volume (μL)	Total volume (μL)	Final concentration (pg/mL)	Remaining volume (μL)
Pre-1	480000000	5	495	500	4800000	490
Pre-2	4800000	10	470	480	100000	475
Pre-3	100000	5	45	50	10000	30
Pre-4	10000	20	180	200	1000	80
7	1000	120	480	600	200	300
6	200	300	300	600	100	300
5	100	300	300	600	50	300
4	50	300	300	600	25	300
3	25	300	300	600	12.5	300
2	12.5	300	300	600	6.25	600
1	/	/	300	300	0	300

Operation procedures

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

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

(3) Loading: Add 10 μL of lysis buffer to each well and then add 90 μL of standard, sample dilution working solution, negative control to the corresponding well. Seal the microplate with microplate sealer and incubate in a 37℃ constant

temperature shaking incubator at 200-300 rpm for 60 minutes.

(4) Plate washing: Discard the liquid in each well, and fill the wells with 1× PBST Wash Buffer (300 µL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on the filter paper after each washing.

(5) Addition of biotinylated detection antibody working buffer: Add 100 µL of biotin detection antibody working solution into each well, seal the microplate with microplate sealer, and incubate in a 37℃ constant temperature shaking incubator at 200-300 rpm for 60 minutes.

(6) Plate washing: Discard the liquid in each well, and fill the wells with 1× PBST Wash Buffer (300 µL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on tissue after each washing.

(7) Addition of enzyme conjugate working buffer: Add 100 µL of enzyme conjugate working buffer to each well. After sealing the plate with a plate sealer, place the plate in a thermostat shaking incubator at 37℃, and incubate for 60 minutes at 200 ~ 300 rpm.

(8) Plate washing: Discard the liquid in each well, and fill the wells with 1× PBST Wash Buffer (300 µL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on tissue after each washing.

(9) Color development: Add 50 µL of substrate Color Reagent A and 50 µL of substrate Color Reagent B to each well, shake gently to mix well, and then seal the plate with microplate sealer at 25℃ for 10 minutes.

(10) Assay: Add 50 µL of Stop Solution to each well and gently shake to mix well. 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.

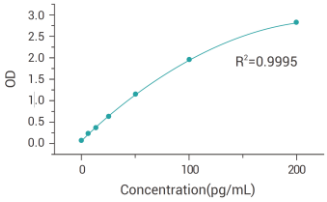
Results process

The 4-parameter fitting method is recommended for the linear fitting and calculation of 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 (pg/mL)	OD value (1)	OD value (2)	Mean value
200	2.846	2.841	2.844
100	1.965	1.979	1.972
50	1.110	1.189	1.150
25	0.636	0.582	0.609
12.5	0.362	0.338	0.350
6.25	0.213	0.204	0.209
0	0.061	0.059	0.060

(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)



Limitations of the assay method

This reagent is only used to detect the content of P24 protein component in the sample.

Precautions

(1) For samples with very high levels of p24 protein: Samples with high levels of p24 (i.e., > 200 pg/mL) must be diluted prior to assay to obtain accurate p24 values. Such samples may include lentiviral supernatant. For crude lentiviral supernatant, it is recommended to dilute by 5000 – 40000 folds, while for finished products, it is recommended to dilute by 40000 – 160000 folds. When testing 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. Diluent should be mixed thoroughly before further analysis or dilution. Analyze each sample in duplicate to determine the correct p24 value in the original sample.

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

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

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

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

(6) Please check each reagent in the kit 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 to absorb the liquid.

(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 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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