TECHNICAL MANUAL

HIV-1 p24 Residual Contamination PharmaGenie ELISA Kit

- **SKU CODE:** AEGE00008
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

Revised: 08/2025

HIV-1 p24 Residual Contamination PharmaGenie ELISA Kit

Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Assay Range:

6.25-200 pg/mL

Limit of quantification:

6.25 pg/mL

Limit of quantification:

3.125 pg/mL

Detection Method:

Sandwich

Sample Type:

Biological samples

Precision:

CV%≤10%, RE%≤±15%

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit as indicated in section 4. Validity for 12 months . Date of expiration is on the ELISA Box label.

3. Product Description

The HIV-1 p24 Residual Contamination PharmaGenie ELISA Kit enables the quantitative detection of HIV-1 p24 protein in biological products. HIV-1 p24, a core capsid protein of the human immunodeficiency virus, can be introduced as a process-related impurity when viral vectors or HIV-derived components are used in gene therapy, vaccine development, or other biopharmaceutical applications. Even trace amounts of residual p24 antigen may indicate incomplete viral clearance, posing potential safety risks and regulatory non-compliance. Sensitive detection of p24 is therefore a critical step in confirming product purity and ensuring patient safety.

This assay employs a highly specific double-antibody sandwich enzyme immunoassay for accurate and reproducible results. Microplate wells are pre-coated with a monoclonal antibody specific to HIV-1 p24. Standards and test samples are added and incubated, allowing any p24 present to bind to the immobilized capture antibody. After washing to remove unbound material, a biotin-labelled anti-p24 detection antibody is added, followed by avidin–HRP, forming an antibody–antigen–biotin–avidin–HRP complex.

The TMB substrate is then introduced, producing a colorimetric change proportional to the amount of HIV-1 p24 in the sample. The reaction is stopped with stop solution, and absorbance is measured at 450 nm. Protein concentration is determined by comparison with the standard curve, providing a sensitive, quantitative method for residual p24 monitoring in compliance with stringent regulatory standards.

4. Kit Contents

No	Component Name	Size	Preparation	Storage
1	Coated microtiter plate	8 wells x 12 strips	Ready-to-use	Store 2 ~ 8°C (Protect from light)
2	Anti-p24-Biotin (detection antibody)	150 µL x 1 vial	1:100, and dilute in 20% CS dilution buffer	
3	Streptavidin HRP (enzyme conjugate)	150 µL x 1 vial 1		
4	HIV-1 p24 Standard	30 µL x 1 vial (0.48 mg/mL)	Operate as per the recommended dilution procedure	
5	Lysis Buffer	1.5 mL x 1 vial	Ready-to-use	
6	20%CS Buffer	25 mL x 1 bottle	Ready-to-use	Store -18°C
7	Sample Diluent Buffer	50 mL x 1 bottle	Ready-to-use	Store 2 ~ 8°C (Protect from light)
8	20xPBST Wash Buffer (20xPBST)	50 mL x 1 bottle	1:20, dilute in deionized water	
9	Color Reagent A	7 mL x 1 bottle	Ready-to-use	
10	Color Reagent B	7 mL x 1 bottle	Ready-to-use	
11	Stop Solution	7 mL x 1 bottle	Ready-to-use	
13	Plate Sealer	1 piece	-	-
14	Technical Manual	1 copies	-	-

Additional materials required:

1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.

5. Precautions

1. Store all reagents according to the instructions on the product label. Before use, allow all reagents to equilibrate to room temperature.
2. Before opening the secondary packaging, bring the pre-coated strip plates to room temperature. Return any unused strips immediately to the original packaging and reseal tightly. Store unused plates at 4°C for up to one month. All other unused reagents should be properly sealed or covered.
3. The volumes of the standard, biotinylated antibody, and enzyme conjugate are small. Perform a quick centrifugation prior to use to ensure that any liquid adhering to the tube walls or caps collects at the bottom.
4. Always use disposable pipette tips during the assay to prevent cross-contamination.
5. Inspect all kit components before use. To ensure accurate results, mix thoroughly when preparing dilutions, loading samples, or adding stop solution.
6. During the washing steps, after removing Wash Buffer, tap the plate dry on clean absorbent paper until no residual droplets or watermarks are visible. Do not insert tissue directly into the wells.
7. The TMB substrate is photosensitive, protect it from prolonged light exposure. Avoid contact with metal surfaces, as this may interfere with the reaction.
8. This kit is intended for single use and should be used within its stated shelf life.

6. Sample Preparation

Due to the inherent variability of biological samples and the specific requirements of individual assays, users are advised to optimize protocols in accordance with their own experimental conditions. Samples may be tested directly with this ELISA or diluted as necessary, based on experimental objectives and the physicochemical characteristics of the sample matrix.

For samples with very high levels of p24 protein (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.

Note: *For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

7. Reagent Preparation

1. **1X PBST Wash Buffer:** Calculate the volume of working solution required for the test, transfer an appropriate amount of 20 x 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.

   Preparation of standard: The standard and test samples should be diluted with the Diluent Buffer. Follow dilutions as below. ***Note:*** *Store the first intermediate gradient (Pre-1) of the standard at -20°C for 1-7 days.*

Vial	Std [pg/mL]	Std vol (µL)	Diluent Buffer (µL)	Total Vol (µL)	Final conc [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	600	0	300

8. Assay Procedure

1. **Equilibrate Reagents:** Bring each component in the kit to room temperature for 30 minutes.
2. **Setup Plate:** Set the standard wells, blank wells and test sample wells, respectively. Recommended to assay samples in duplicate.
3. **Add Standard, Sample and Blanks:** 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.
4. **First Incubation:** Seal the microplate with microplate sealer and incubate in a 37°C constant temperature shaking incubator at 200-300 rpm for 60 minutes.
5. **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.
6. **Addition of enzyme conjugate working buffer:** Add 100 µL of enzyme conjugate working buffer to each well.
7. **Second Incubation:** Seal the microplate with microplate sealer and incubate in a 37°C constant temperature shaking incubator at 200-300 rpm for 60 minutes.

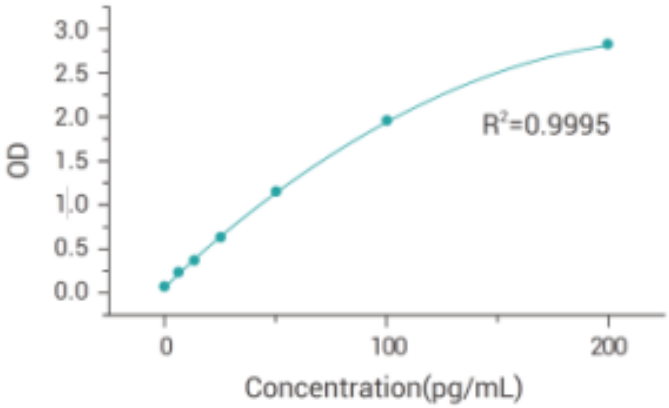
8. **Washing:** Repeat step 5.
9. **Colour development:** Add 50 µL of substrate Color Reagent A and 50 µL of substrate Colour Reagent B to each well, shake gently to mix well, and then seal the plate with microplate sealer at 25°C. for 10 minutes.
10. **Reaction termination**: Add 100 µL of stop solution to each well and immediately perform the detection.
11. **Signal Reading**: Measure the OD values at 450 nm and 630 nm with a plate reader. The measurement should be completed within 30 minutes after reaction termination.

9. Data Analysis

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

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)


3.0
2.5
2.0
1.5
1.0
0.5
0.0
OD
R²=0.9995
0
100
200
Concentration(pg/mL)

10. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.