HUABIO

HUABIO RESEARCH INC

Lentivirus HIV-1 p24 Instructions

Cat: EHY0002

Content

	CAT	Volume
1 CP (Coated Plate)	EHY0002CP	96 well
2 S (Standard)	EHY0002S1~S7,S0	8 vial
3 DA-H (Detect Antibody-HRP)	EHY0002DA-H	6 ml/bottle
4 SD (Sample Diluent)	ESD01	15 ml/bottle
5 VL (Virus Lysis)	EVL01	12 ml/bottle
6 TS (TMB Substrate)	ETS01	12 ml/bottle
7 SS (Stop Solution)	ESS01	12 ml/bottle
8 WB (Wash Buffer 10×)	EWB01	50 ml/bottle
9 SF (Sealer Film)	ESF01	6 pieces

■ **NOTE:** After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

Sample Dilution

Samples of the crude virus or filtered need a 20-fold dilution into Sample Diluent. A suggested 20-fold is 10 μl of sample + 190 μl of Sample Diluent.

For chromatographic samples, at least 100-fold dilution is required. A suggested 100-fold is 5 μl of sample + 495 μl of Sample Diluent.

REAGENT PREPARATION

■ **Washing Buffer (1×) Preparation**

Pour entire contents (50 ml) of the **Washing Buffer Concentrate** (10×) into a clean 500 ml graduated cylinder. Bring to final volume of 500 ml with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25℃.

■ **Standard Curve Preparation:**

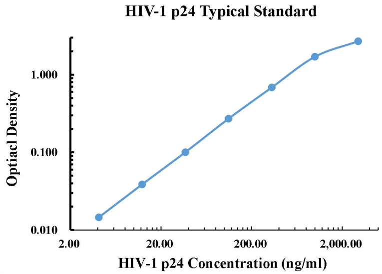
S1 to S7 and S0 is ready to use.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess **CP** (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.
3. Add 50 μl of **VL** (Virus Lysis) to each well.
4. Add 10 μl of **Standard or sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.
5. Add 50 μl of **DA-H** (Detect Antibody-HRP) to each well.
6. Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 30 mins on a microplate **shaker** set at 500 rpm.
7. Aspirate each well and **wash**, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300 μl). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining WB (Washing Buffer) by aspirating or decanting. Invert the plate and **blot** it against clean paper towels.
8. Add 100 μl of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.
9. Add 100 μl of **SS** (Stop Solution) to each well.
10. Determine the optical density within 30 minutes, using microplate **reader** set to 450 nm corrected with 570 nm or 630 nm.

TYPICAL DATA



ng/ml	O.D.		Average	Corrected
0.00	0.0072	0.0068	0.0070	
4.12	0.0220	0.0212	0.0216	0.0146
12.35	0.0453	0.0461	0.0457	0.0387
37.04	0.1085	0.1066	0.1076	0.1006
111.11	0.2794	0.2775	0.2785	0.2715
333.33	0.6978	0.6874	0.6926	0.6856
1000.00	1.7410	1.6832	1.7121	1.7051
3000.00	2.7224	2.6821	2.7023	2.6953

SENSITIVITY

The minimum detectable dose (MDD) of HIV-1 p24 is typically less than 0.12 ng/ml.

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

PRECISION

■ **Intra-assay Precision (Precision within an assay)** Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

■ **Inter-assay Precision (Precision between assays)**

	Intra-assay Precision				Inter-assay Precision		
Sample Number	S1	S2	S3		S1	S2	S3
	22	22	22		6	6	6
Average (ng/ml)	62.5	333.8	928.4		62.6	324.3	978.5
Standard deviation	3.1	15.9	58.3		1.8	6.0	39.4
Coefficient of variation (%)	4.9	4.8	6.3		3.9	1.9	4.0

RECOVERY

The spike recovery was evaluated by spiking 3 levels of HIV-1 p24. The recovery ranged from 95% to 106% with an overall mean recovery of 101%.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of HIV-1 p24. The linearity ranged from 97% to 103% with an overall mean recovery of 100%.