

# Quantikine™ ELISA

## HIV-1 Gag p24 Immunoassay

Catalog Number DHP240B

For the quantitative determination of HIV-1 Gag p24 concentrations in cell culture supernates.

This package insert must be read in its entirety before using this product.  
For research use only. Not for use in diagnostic procedures.

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## INTRODUCTION

HIV-1 Gag p24, also known as the capsid protein (CA), is essential for HIV-1 viral replication and the ability of HIV-1 to infect non-dividing cells (1). The mature HIV-1 viral capsid is composed of approximately 1100 Gag p24 monomers assembled into a lattice that encapsulates the viral genome (2, 3). Peptides derived from Gag p24 antigen processing can be presented on infected cells in association with HLA-C molecules. Recognition of these complexes by the inhibitory KIR2DL2 on NK cells enables the survival of infected cells by suppressing NK cell-mediated target cell cytotoxicity (4). Gag p24 concentration in the plasma is commonly used as an indicator of viral load (5-7). The development of anti-Gag p24 humoral responses following viral infection leads to immune complex formation and reduction of the amount of free Gag p24 in circulation (8).

The Quantikine™ HIV-1 Gag p24 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure HIV-1 Gag p24 in cell culture supernates.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for HIV-1 Gag p24 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any HIV-1 Gag p24 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human HIV-1 Gag p24 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of HIV-1 Gag p24 bound in the initial step. The color development is stopped and the intensity of the color is measured.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine™ Immunoassay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
HIV-1 Gag p24 Microplate	894784	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for HIV-1 Gag p24.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
HIV-1 Gag p24 Standard	894786	2 vials of HIV-1 Gag p24 in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Use a fresh standard for each assay. Discard after use.
HIV-1 Gag p24 Conjugate	894785	21 mL of a monoclonal antibody specific for HIV-1 Gag p24 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-124	896059	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. <i>Use diluted 1:4 in this assay.</i>	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives. <i>May turn yellow over time.</i>	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

\* Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 50 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples
- HIV-1 Gag p24 Controls (optional; R&D Systems®, Catalog # QC221)

## PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage condition listed below is intended as a general guideline. Sample stability has not been evaluated.**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

## SAMPLE PREPARATION

Cell culture supernate samples require a 2-fold dilution due to a matrix effect. A suggested 2-fold dilution is 150  $\mu$ L of sample + 150  $\mu$ L of Calibrator Diluent RD5-26 (diluted 1:4)\*.

\*See Reagent Preparation section.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

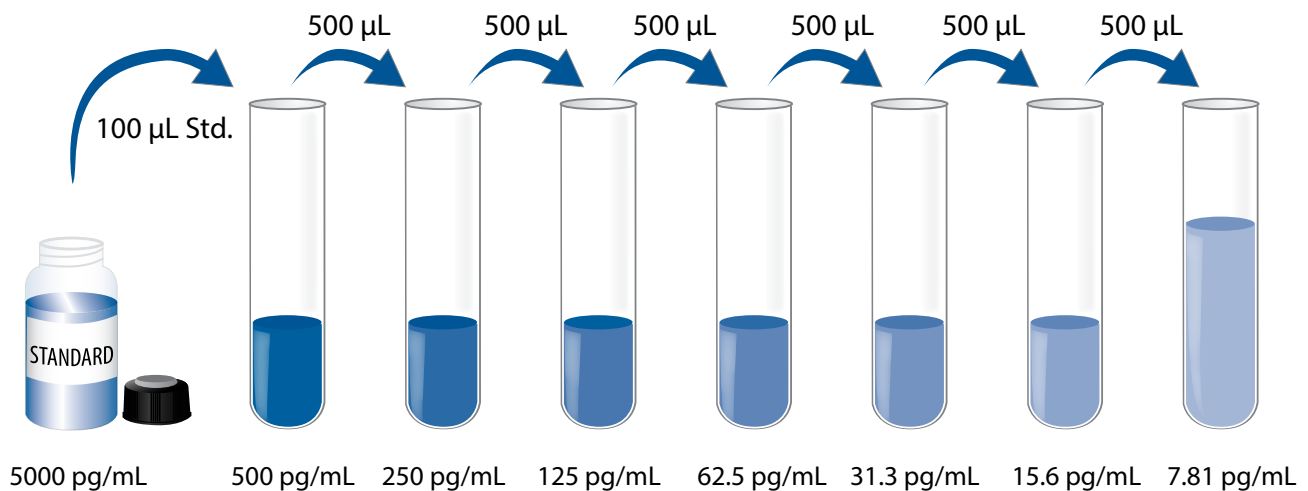
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200  $\mu$ L of the resultant mixture is required per well.

**Calibrator Diluent RD5-26 (diluted 1:4)** - Add 10 mL of Calibrator Diluent RD5-26 Concentrate to 30 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5-26 (diluted 1:4).

**HIV-1 Gag p24 Standard - Refer to the vial label for reconstitution volume.** Reconstitute the HIV-1 Gag p24 Standard with deionized or distilled water. This reconstitution produces a stock solution of 5000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

Pipette 900  $\mu$ L of Calibrator Diluent RD5-26 (diluted 1:4) into the 500 pg/mL tube. Pipette 500  $\mu$ L into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 pg/mL).



## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.**

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100  $\mu\text{L}$  of Assay Diluent RD1-124 to each well.
4. Add 100  $\mu\text{L}$  of standard, control, or sample\* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at  $500 \pm 50$  rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400  $\mu\text{L}$ ) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200  $\mu\text{L}$  of HIV-1 Gag p24 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200  $\mu\text{L}$  of Substrate Solution to each well. **Protect from light.** Incubate for 30 minutes at room temperature.
9. Add 50  $\mu\text{L}$  of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

\*Samples may require dilution. See Sample Preparation section.



## CALCULATION OF RESULTS

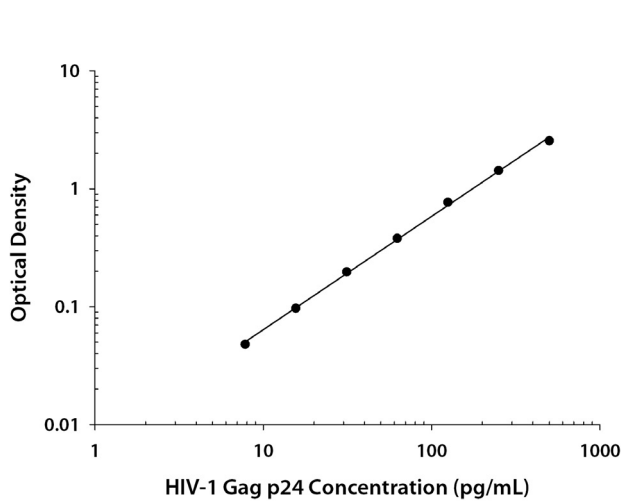
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the HIV-1 Gag p24 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.056 0.058	0.057	—
7.81	0.105 0.105	0.105	0.048
15.6	0.152 0.155	0.154	0.097
31.3	0.251 0.259	0.255	0.198
62.5	0.428 0.447	0.438	0.381
125	0.816 0.835	0.826	0.769
250	1.480 1.496	1.488	1.431
500	2.574 2.658	2.616	2.559

## 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)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	45.7	135	280	43.0	132	285
Standard deviation	1.41	4.86	8.10	1.93	4.61	11.2
CV (%)	3.1	3.6	2.9	4.5	3.5	3.9

## RECOVERY

The recovery of HIV-1 Gag p24 spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	100	93-105%

## SENSITIVITY

Thirty assays were evaluated and the minimum detectable dose (MDD) of HIV-1 Gag p24 ranged from 0.240-3.25 pg/mL. The mean MDD was 1.03 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

## CALIBRATION

This immunoassay is calibrated against highly purified native HIV-1 Gag p24 protein.

The NIBSC/WHO HIV-1 p24 Antigen International Standard 90/636 was evaluated in this kit. The dose response curve of the standard 90/636 parallels the Quantikine™ standard curve. To convert sample values obtained with the Quantikine Human HIV-1 Gag p24 kit to approximate NIBSC/WHO 90/636 Units, use the equation below.

NIBSC/WHO (90/636) approximate value (IU/mL) = 0.25 x Quantikine HIV-1 Gag p24 value (pg/mL)

**Note:** Based on data generated in August 2015.

## LINEARITY

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

		Cell culture media (n=4)
1:2	Average % of Expected	96
	Range (%)	93-98
1:4	Average % of Expected	93
	Range (%)	87-97
1:8	Average % of Expected	92
	Range (%)	82-110
1:16	Average % of Expected	86
	Range (%)	83-88

## SAMPLE VALUES

### Cell Culture Supernates -

LAV/8E5 human immunodeficiency virus was cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and gentamycin. An aliquot of the cell culture supernate was removed, assayed for HIV-1 Gag p24, and measured 13,680 pg/mL.

## SPECIFICITY

This assay recognizes HIV-1 Gag p24.

The factors listed below were prepared in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors in a mid-range HIV-1 Gag p24 control were assayed for interference. No significant cross-reactivity or interference was observed.

### Purified proteins:

HTLV-1 p24

HTLV-2 p24

SIV p27

## REFERENCES

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