Quantikine® ELISA

HIV-1 Gag p24 Immunoassay

Catalog Number DHP240

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

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, serum, and plasma.

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 standard 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.

<table>
<thead>
<tr>
<th>PART</th>
<th>PART #</th>
<th>DESCRIPTION</th>
<th>STORAGE OF OPENED/RECONSTITUTED MATERIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 Gag p24 Microplate</td>
<td>894784</td>
<td>96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for HIV-1 Gag p24.</td>
<td>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.*</td>
</tr>
<tr>
<td>HIV-1 Gag p24 Conjugate</td>
<td>894785</td>
<td>21 mL of a monoclonal antibody specific for HIV-1 Gag p24 conjugated to horseradish peroxidase with preservatives.</td>
<td></td>
</tr>
<tr>
<td>Assay Diluent RD1-124</td>
<td>896059</td>
<td>11 mL of a buffered protein base with preservatives.</td>
<td></td>
</tr>
<tr>
<td>Calibrator Diluent RDS-26 Concentrate</td>
<td>895525</td>
<td>21 mL of a concentrated buffered protein base with preservatives. Use diluted 1:4 in this assay.</td>
<td>May be stored for up to 1 month at 2-8 °C.*</td>
</tr>
<tr>
<td>Wash Buffer Concentrate</td>
<td>895003</td>
<td>21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives. May turn yellow over time.</td>
<td></td>
</tr>
<tr>
<td>Color Reagent A</td>
<td>895000</td>
<td>12 mL of stabilized hydrogen peroxide.</td>
<td></td>
</tr>
<tr>
<td>Color Reagent B</td>
<td>895001</td>
<td>12 mL of stabilized chromogen (tetramethylbenzidine).</td>
<td></td>
</tr>
<tr>
<td>Stop Solution</td>
<td>895032</td>
<td>6 mL of 2 N sulfuric acid.</td>
<td></td>
</tr>
<tr>
<td>Plate Sealers</td>
<td>N/A</td>
<td>4 adhesive strips.</td>
<td></td>
</tr>
</tbody>
</table>

* 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).
- Sample Activation Kit 1 (R&D Systems, Catalog # DY010).
**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. Please refer to the MSDS on our website prior to use.

**SAMPLE COLLECTION & STORAGE**

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:** Citrate plasma has not been validated for use in this assay. Grossly icteric samples are not suitable for use in this assay.

**SAMPLE PREPARATION**

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

Serum and plasma samples may be run neat.

For immune complex dissociation in serum and plasma samples, add 120 μL of sample + 60 μL of 1N HCl and mix well. Incubate for 10 minutes at room temperature. Add 60 μL of 1.2N NaOH + 0.5M Hepes and mix well. Total sample dilution due to ICD protocol is 1:2. ICD treatment buffers are sold as Sample Activation Kit 1 (R&D Systems, Catalog # DY010).

*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 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 μL 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 μL of Calibrator Diluent RD5-26 (diluted 1:4) into the 500 pg/mL tube. Pipette 500 μL 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).

<table>
<thead>
<tr>
<th>Concentration (pg/mL)</th>
<th>Volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5000</td>
<td>500</td>
</tr>
<tr>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>125</td>
<td>500</td>
</tr>
<tr>
<td>62.5</td>
<td>500</td>
</tr>
<tr>
<td>31.3</td>
<td>500</td>
</tr>
<tr>
<td>15.6</td>
<td>500</td>
</tr>
<tr>
<td>7.81</td>
<td>500</td>
</tr>
</tbody>
</table>

**Diagram:**
- 100 μL Std. → 500 μL → 500 μL → 500 μL → 500 μL → 500 μL → 500 μL
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls 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 μL of Assay Diluent RD1-124 to each well.

4. Add 100 μL 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 ± 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 μL) 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 μL 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 μL of Substrate Solution to each well. Protect from light. Incubate for 30 minutes at room temperature.

9. Add 50 μL 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.

<table>
<thead>
<tr>
<th>Concentration (pg/mL)</th>
<th>O.D.</th>
<th>Average</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.056 0.058</td>
<td>0.057</td>
<td>—</td>
</tr>
<tr>
<td>7.81</td>
<td>0.105 0.105 0.058</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.6</td>
<td>0.152 0.155</td>
<td>0.154</td>
<td>0.097</td>
</tr>
<tr>
<td>31.3</td>
<td>0.251 0.259 0.154 0.198</td>
<td></td>
<td></td>
</tr>
<tr>
<td>62.5</td>
<td>0.428 0.447 0.255 0.381</td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>0.816 0.835 0.438 0.769</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>1.480 1.496 0.826 1.431</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>2.574 2.616 2.559</td>
<td>2.658</td>
<td></td>
</tr>
</tbody>
</table>
**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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intra-Assay Precision</th>
<th>Inter-Assay Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (pg/mL)</td>
<td>45.7</td>
<td>135</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.41</td>
<td>4.86</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.1</td>
<td>3.6</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture media (n=4)</td>
<td>100</td>
<td>93-105%</td>
</tr>
<tr>
<td>Serum (n=4)</td>
<td>79</td>
<td>73-85%</td>
</tr>
<tr>
<td>EDTA plasma (n=4)</td>
<td>74</td>
<td>64-82%</td>
</tr>
<tr>
<td>Heparin plasma (n=4)</td>
<td>72</td>
<td>63-78%</td>
</tr>
<tr>
<td>Serum, ICD treated (n=4)</td>
<td>91</td>
<td>71-99%</td>
</tr>
<tr>
<td>EDTA plasma, ICD treated (n=4)</td>
<td>92</td>
<td>85-100%</td>
</tr>
<tr>
<td>Heparin plasma, ICD treated (n=4)</td>
<td>88</td>
<td>69-94%</td>
</tr>
</tbody>
</table>

**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 optical density 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.

\[
\text{NIBSC/WHO (90/636) approximate value (IU/mL) = 0.25 x Quantikine HIV-1 Gag p24 value (pg/mL)}
\]

**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.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Cell culture media (n=4)</th>
<th>Serum (n=4)</th>
<th>EDTA plasma (n=4)</th>
<th>Heparin plasma (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>Average % of Expected</td>
<td>96</td>
<td>100</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>Range (%)</td>
<td>93-98</td>
<td>97-104</td>
<td>103-109</td>
</tr>
<tr>
<td>1:4</td>
<td>Average % of Expected</td>
<td>93</td>
<td>101</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>Range (%)</td>
<td>87-97</td>
<td>98-103</td>
<td>105-111</td>
</tr>
<tr>
<td>1:8</td>
<td>Average % of Expected</td>
<td>92</td>
<td>97</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>Range (%)</td>
<td>82-110</td>
<td>94-101</td>
<td>100-103</td>
</tr>
<tr>
<td>1:16</td>
<td>Average % of Expected</td>
<td>86</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Range (%)</td>
<td>83-88</td>
<td>94-102</td>
<td>96-105</td>
</tr>
</tbody>
</table>

**SAMPLE VALUES**

**Serum/Plasma** - Thirty-six samples from apparently healthy volunteers were evaluated for the presence of HIV-1 Gag p24 in this assay. No medical histories were available for the donors used in this study. No detectable levels were observed.

**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.

**ICD TREATMENT DATA**

The recovery of HIV-1 Gag p24 from a synthetic immune complex treated with the ICD protocol throughout the range of the assay in various matrices was evaluated. Sample were ICD treated prior to assay as described in the Sample Preparation section.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (n=4)</td>
<td>104</td>
<td>100-112%</td>
</tr>
<tr>
<td>EDTA plasma (n=4)</td>
<td>105</td>
<td>99-113%</td>
</tr>
<tr>
<td>Heparin plasma (n=4)</td>
<td>106</td>
<td>106-106%</td>
</tr>
</tbody>
</table>
**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**