NOVATEINBIO
Accelerates Research and Development

HIV-1 Glycoprotein 120 (gp120) ELISA Kit

Catalog number: NS-E10006 (96 wells)

The kit is designed to detect the level of HIV gp120 in cell culture supernatant, serum, plasma and other suitable sample solution

FOR **RESEARCH USE ONLY**. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

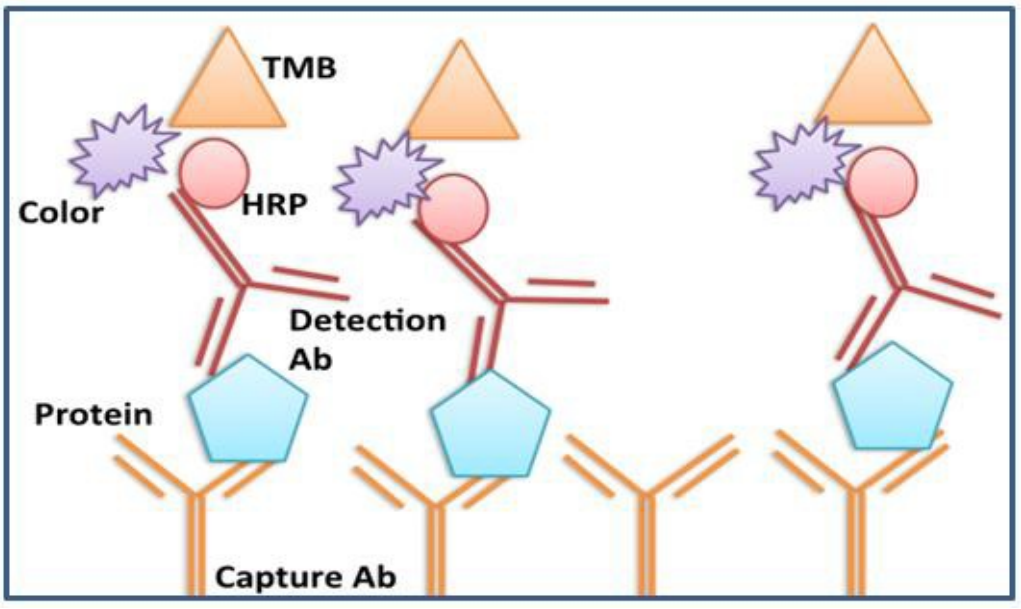
Intended use

The kit is used to quantify the HIV gp120 in cell culture supernatant, serum, plasma and other suitable sample solution.

Standard range	46.8 – 3000 pg/ml
Sensitivity	20 pg/ml
Assay time	5 hours
Validity	Six months
Store at	2-8 °C

Assay principle

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to analyze the level of HIV gp120 in samples. Add standard and sample to wells pre-coated with one HIV gp120 antibody. Wash the plate after the incubation. Add secondary HRP-conjugated HIV gp120 antibody to bind the analyte, followed by incubation and washing procedures to remove unbound substance. Finally, HRP substrates are added, incubated for detection, and a blue color is developed. Reaction is stopped and color turns to yellow when Stopping Solution (acidic) is added. The yellow color intensity proportionally correlates to the concentration of the HIV gp120 in samples.



Materials supplied

1	Microelisa Stripplate	96 well
2	Standard	2 (3.0 ng/vial)
3	Detection antibody	1 vial (1:100, 120μL)
4	20 X Wash Solution	25 ml
5	Sample diluent	10 ml × 2
6	Detection antibody dilution buffer	12 ml
7	Chromogenic Solution A	6 ml
8	Chromogenic Solution B	6 ml
9	Stop Solution	6 ml
10	Plate sealer	2
11	Package insert	1

Materials required but not supplied

37℃ incubator.

Standard plate reader capable of measuring absorbance at 450 nm.
Adjustable pipettes and disposable pipette tips.
Distilled water.
Multi-channel pipettes, manifold dispenser or automated microplate washer.
Absorbent paper.
Materials used for sample preparation.

Sample collection and storage

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before a centrifugation for 15minutes at approximately 1000 x g. Remove serum and perform the assay immediately or aliquot and store samples at -20 °C or -80°C.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30minutes of collection. Store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.

Cell culture fluid and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

NOTE: Serum, plasma, and cell culture fluid samples to be used within 7 days may be stored at 2-8°C, otherwise samples must be stored at -20°C(≤2months) or -80°C (≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES**.

Sample Preparation

Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.

Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.

Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

Standard

HIV gp120: Standard solution should be prepared no more than 2 hours prior to the experiment.

Two tubes of standard (3ng/vial) are included in each kit. Use one tube for each experiment.

3000 pg/ml→46.8 pg/ml of HIV gp120 standard solutions:

Add 1.0 ml of Antibody detection buffer into one standard tube. Keep the tube at room temperature for 10 minutes and mix thoroughly. This is 3000pg/ml standard solution. Label 6 Eppendorf tubes with 1500pg/ml, 750pg/ml, 375pg/ml, 187.5pg/ml, 93.7pg/ml and 46.8 pg/ml, respectively. Aliquot 0.25 ml of the sample diluents into each 6 tubes. Transfer 250 µl from 3000pg/ml to 1500pg/ml tube. Transfer 0.25 ml from 1500pg/ml tube to 750 pg/ml tube and mix. Transfer 0.25 ml from 750pg/ml tube to 375pg/ml tube and mix, and so on.

Note: The standard solutions are best used within 2 hours.

Preparation of Detection antibody working solution

The solution should be prepared no more than 2 hours prior to the experiment.

Detection antibody should be diluted in 1:1000 with the detection antibody dilution buffer and mixed thoroughly.

Wash Solution

Dilute 25 mL of Wash Solution concentrate (20×) with 475 mL of deionized or distilled water to prepare 500 mL of Wash Solution (1×).

Assay procedures

1. Prepare all the Standards before starting assay procedure (Please read Reagents Preparation). It is recommended that all Standards and Samples should be added in duplicate to the Microtiter Plate.
2. Secure the desired numbers of coated wells in the holder. Take the Standards and agitate gently prior to use then add 100µl of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate.
3. Add 100µl sample diluent to the blank wells. Seal the plate and incubate for 2 hours at room temperature.
4. Aspire each well and wash with at least 300 µl of wash buffer. Repeat the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
5. Add 100µl of diluted detection antibody to each well. Mix well. **Mixing well in this step is important.** Cover and incubate the plate for 1 hour at room temperature.
6. Repeat the aspiration&wash as in step 4.
7. Add 50µl Chromogenic Substrate A and 50µl Chromogenic Substrate B to each well, subsequently. Cover and incubate for 20-60 minutes at 20-25°C. (Protect from light. Do not over develop).
8. Add 50µl Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

Important notes

The operation should be carried out in strict accordance with the provided instructions.

Store the unused strips in a sealed foil bag at 2-8°C.

Always avoid foaming when mixing or reconstituting protein solutions.

Pipette reagents and samples into the center of each well, avoid from bubbles.

The samples should be transferred into the assay wells within 15 minutes of dilution.

We recommended that all standard, testing samples are tested in duplicate.

Use serial diluted sample is recommended for first test to get the best dilution factor.

If the blue color develops too shallow after 40 minutes incubation with the substrates, it may be appropriate to extend the incubation time. (Do not over develop)

Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.

Avoid using the suction head without extensive wash.

Do not mix the reagents from different batches.

Stop Solution should be added in the same order of the Substrate solution.

Chromogenic Solution B is light-sensitive. Avoid prolonged exposure to the light.

Result calculation

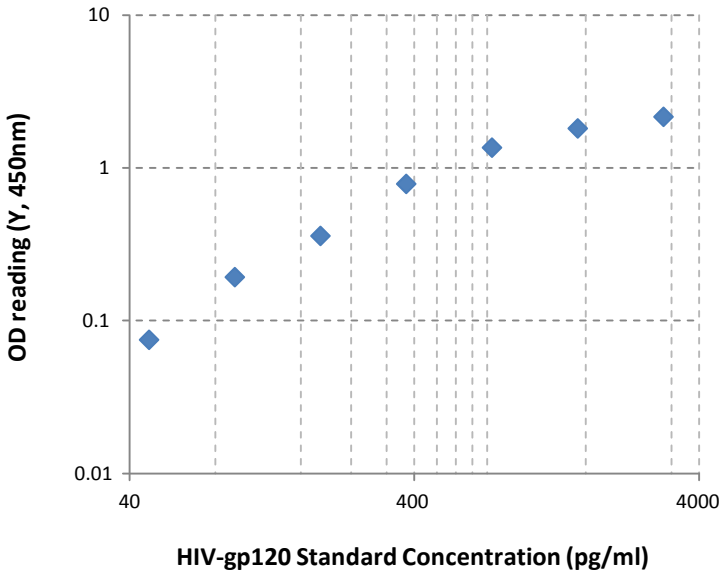
The standard curve is used to determine the amount of samples.

First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the blank control before result interpretation.

Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and draw a best fit curve e using graph paper or statistical software to generate a four paramater logistic (4-PL) curve-fit or logit-log linear regression curve. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.

Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. A standard curve should be generated for each assay.

Typical data:



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