Human HIV (antibodies plus p24 antigen) HIV-P24 ELISA Kit
CatalogueNumber: OM527099
(96Tests)
Store all reagents at 2-8°C
Collect sample: serum or blood plasma

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

INTENDED USE

This HIV-P24 ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures.The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of HIV-P24 in the sample, this HIV-P24 ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a cutoff value. The existence or not of HIV-P24 in the samples is then determined by comparing the O.D. of the samples to the CUT OFF.

PRINCIPLE OF THE ASSAY

The kit assay HIV-P24 level in the sample， use Purified antigen to coat microtiter plate wells, make solid-phase antigen, then add HIV-P24 to wells, Combined With HIV-P24, after washing and removing non-combinative antibody and other components ,then Combined antigen which with HRP labeled become antigen - antibody - enzyme- antigen complex, after washing Completely, Add TMB substrate solution,, TMB Chromogen Solution Becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. Compared with the CUTOFF value, according to this to judge HIV-P24 exist in the sample or not.

REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

1. MICROTITER PLATE 96 wells
2. Sample diluent 6.0 mL 1 tube
3. Negative control 0.5ml 1 tube
4. Positive control 0.5ml 1 tube
5. STREPTAVIDIN-HRP 6.0 mL 1 tube
6. Chromogen Solution A 6.0 mL 1 vial
7. Chromogen Solution B 6.0 mL 1 vial
8. STOP SOLUTION 6.0 mL 1 vial
9. WASH SOLUTION x30 20 mL 1 vial
10. Instruction 1

SAMPLE COLLECTION AND STORAGE

Serum- Use a serum separator tube(SST) and allow samples to clot for 30minutes before centrifugation for 15minutes at approximately 1000 x g.Remove serum and 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 stored at -20°C(≤2months) or -80°C(≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles .When performing the assay slowly bring samples to room temperature.

DO NOT USE HEAT-TREATED SPECIMENS.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 ml to 1 ml volumes. .
3. Adjustable 10ml -100ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
6. Absorbent paper.
7. 37°C incubator.
8. Distilled or deionized water.

9. Data analysis and graphing software..
10. Tubes to prepare standard or sample dilutions.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from Human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate viruses.
10. Solid Waste: Autoclave 60 min. at 121°C.
11. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
12. Substrate Solution is easily contaminated. If bluish prior to use, do not use.
13. Chromogen Solution B contains 20% acetone, keep this reagent away from sources of heat or flame.
14. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C).

REAGENT PREPARATION

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20mL Wash Buffer Concentrate into deionized or distilled water to prepare 600mL of Wash Buffer.

ASSAY PROCEDURE

Prepare all Standards before starting assay procedure (see Preparation Reagents). It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.

1. Number: to sample correspond microtitration well and Number Sequence, each plate should be set feminine comparison 2 wells, masculine comparison 2 wells, blank comparison 1 well(don't add sample and HRP-Conjugate reagent to blank comparison well, other each step the operation are same).

2. Add sample: separately add Positive control and Negative control 50 μl to the Positive and Negative well . add Sample dilution 40 μl to testing sample well, then add testing sample 10 μl. add sample to the bottom of ELISA plates coated well , don't touch the well wall as far as possible, and Gently mix. 3.Incubate: After closing plate with Closure plate membrane ,incubate for 30 min at 37℃.

4. Configurate liquid: 30-fold (or 20-fold)wash solution diluted 30-fold (or 20-fold) with distilled water until 600ml,and reserve.

5. Washing: Uncover Closure plate membrane, discard Liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat.

6. Add enzyme: Add HRP-Conjugate reagent 50 μl to each well, except the blank well.

7. Incubate: Operation with 3.

8. Washing: Operation with 5.

9. Color: Add Chromogen Solution A 50ul and Chromogen Solution B 50ul to each well, evade the light preservation for 15 min at 37℃

10. Stop the reaction: Add Stop Solution50 μl to each well, Stop the reaction(the blue color change to yellow color).

11. Assay: take blank well as zero , Read absorbance at 450nm after Adding Stop Solution and within 15min.

TYPICAL DATA

Determine the result

Test validity: the average of Positive control well≥1.00; the average of Negative control well ≤0.10.

Calculate Critical(CUT OFF) : Critical= the average of Negative control well + 0.15.

Negative control: sample OD< Calculate Critical(CUT OFF) is HIV-P24 Negative control.

Positive control: sample OD≥ Calculate Critical(CUT OFF) is HIV-P24 Positive control.