

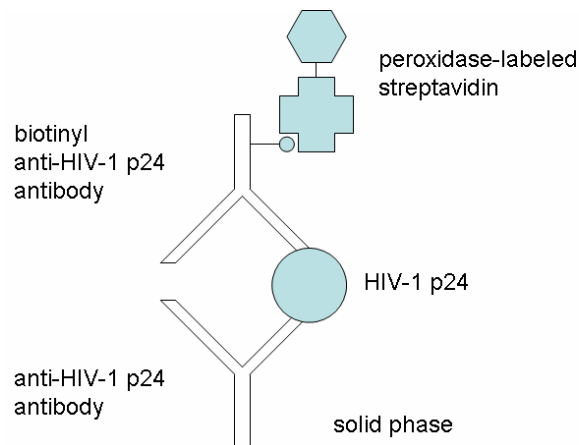
HIV-1 p24 ELISA Kit “BioAcademia”

80-001 1 kit 96 assays

This kit can measure the amount of HIV-1 Gag p24 antigen in cell culture medium handily by a sandwich ELISA (Enzyme Linked Immunosorbent Assay) method. p24 antigen is a structure protein of HIV-1, so when this is measured, it's possible to presume the virus amount in the sample.

[Principle of the test]

This kit is made based on the principle of a sandwich ELISA method using an antibody coated plate as a solid phase, a biotinyl antibody and a peroxidase-labeled streptavidin.



[Advantage of this kit]

- **p24 of subtype AE can be measured** at the same sensitivity as subtype B, because affinity purified polyclonal antibody raised against the full length recombinant p24 is used.
- **Risk-free kit**, because neither patient sera nor active virus products are used.
- **Assay can be performed at room temperature.**

[Preservation and Expiration Date]

Storage : 2 – 8°C (Please don't freeze.)

Expiration date for use : 1 year

[Required Reagent, Apparatus and Equipment]

1. Deionized water
2. Test tubes or microtubes (for sample preparation)
3. Micropipettes and tips
4. Microplate reader

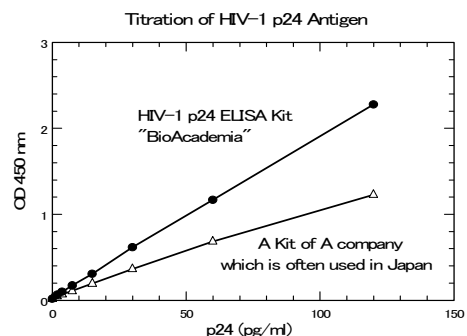


Figure Standard curve of HIV-1 p24 measurement by the 2 hour assay.

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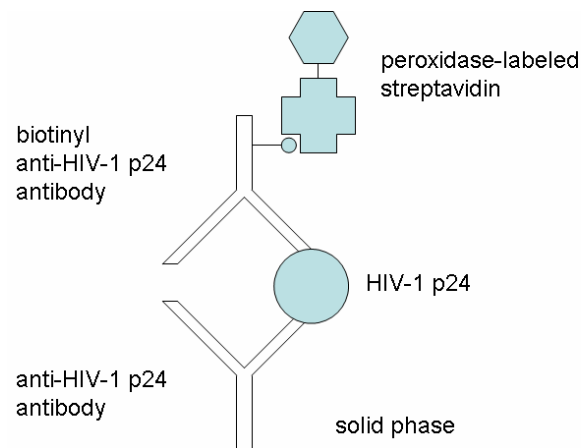
Instruction Manual

[Usage]

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[Principle of the test]

This kit is made based on the principle of a sandwich ELISA method using an antibody coated plate as a solid phase, a biotinyl antibody and a peroxidase-labeled streptavidin.



[Materials]

- | | |
|---|----------------------|
| 1. Antigen Standard : recombinant HIV-1 p24 (120 pg/ml) | 3 tubes (1.0 ml x 3) |
| 2. Antigen Diluent : phosphate buffer | 1 bottle (25 ml) |
| 3. Sample Buffer : 10% Triton X-100 | 1 bottle (25 ml) |
| 4. Wash Buffer : phosphate buffer (Please dilute 20-fold.) | 1 bottle (30 ml) |
| 5. Antibody Coated Plate : anti-HIV-1 p24 antibody-coated microplate | 8 wells x 12 strips |
| 6. Biotinyl Antibody : biotinyl anti-HIV-1 p24 antibody (101-fold concentrated solution.
An animal serum is included.) | 1 bottle (200 µl) |
| 7. Biotinyl Antibody Diluent : phosphate buffer, 2% casein | 1 bottle (15 ml) |
| 8. Enzyme-Labeled (101-fold concentrated solution): peroxidase-labeled streptavidin | 1 tube (200 µl) |
| 9. Enzyme-Labeled Diluent : HEPES buffer, 1% BSA | 1 bottle (15 ml) |
| 10. Substrate Solution : 3,3',5,5'-tetramethylbenzidine/H ₂ O ₂ | 1 bottle (20 ml) |
| 11. Stop Solution : 0.5 M sulfuric acid | 1 bottle (20 ml) |
| 12. Plate Sealer : | 3 seats |

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[Test Procedure]

Step 1

- 1) Dilute Antigen Standard (120 pg/ml) with Antigen Diluent. The ideal concentrations are 0, 7.5, 15, 30, 60 pg/ml.
- 2) Add 1/10 volume of Sample Buffer to specimen for the isolation of p24 antigen from HIV-1, and then dilute with Antigen Diluent to 10 – 100 pg/ml of antigen concentration.

Step 2

- 1) Wash each well of Antibody Coated Plate with 350 μ l of 20-fold diluted Wash Buffer twice.
- 2) Add 200 μ l of the diluted Antigen Standard or specimen solutions into the washed wells, and incubate at 37°C* for 2 hours.
- 3) Remove the solutions in the wells by aspiration, and wash the wells with 350 μ l of 20-fold diluted Wash Buffer three times**.

Step 3

- 1) Dilute Biotinyl Antibody 101-fold with Biotinyl Antibody Diluent.
- 2) Add 100 μ l of the diluted Biotinyl Antibody into the washed wells, and incubate at 37°C* for 1 hour.
- 3) Remove the solutions in the wells by aspiration, and wash the wells with 350 μ l of 20-fold diluted Wash Buffer three times**.

Step 4

- 1) Dilute Enzyme-Labeled 101-fold with Enzyme-Labeled Diluent.
- 2) Add 100 μ l of the diluted Enzyme-Labeled into the washed wells, and incubate at 37°C* for 30 minutes.
- 3) Remove the solutions in the wells by aspiration, and wash the wells with 350 μ l of 20-fold diluted Wash Buffer three times**.

Step 5

- 1) Add 100 μ l of Substrate Solution into the washed wells, and incubate at room temperature for 30 minutes.
- 2) Add 100 μ l of Stop Solution into the wells, and read the optical density at 450 nm of the wells using a microplate reader within 10 minutes.

* : Please incubate at 37°C during all procedure except for step 5. (The incubations are also possible at room temperature, but the coloring level becomes low).

** : Three times are usually enough for washing, but if the measure of antigen amount 0 isn't fixed, please increase the washing number of times after the incubations.

***: Please use Plate Sealer supplied at long incubation.

[Precautions]

1. This kit is for research only and it can't be used for diagnostic use.
2. A user has to consider and treat infectibility samples safely.
3. A user must be careful about handling and disposal of Stop Solution sufficiently, because it is strong acid.

[Reference]

1. White, E. L., et al., J. Virol. Methods 70: 113-115 (1998)

<Distributed by>

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