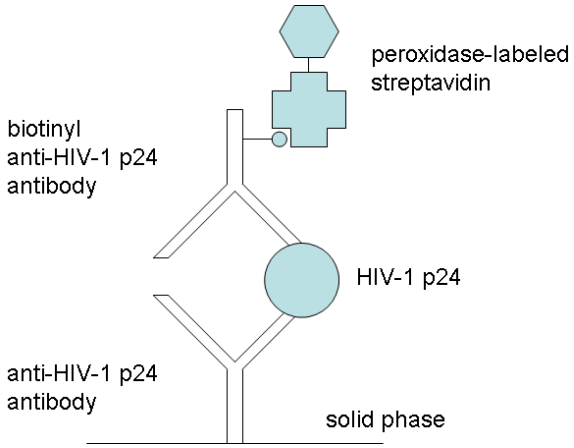
# HIV-1 p24 ELISA Kit "BioAcademia"

80-001　　1 kit 96 assays

This kit is used to measure handily the amounts of HIV-1 Gag p24 antigen in cell culture medium and of the lentivirus vectors derived from HIV-1 by a sandwich ELISA (Enzyme Linked Immunosorbent Assay) method. Since p24 antigen is a structure protein of HIV-1, the virus amounts in the sample can be measured from the value of p24 titration.

**[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℃ (Please don't freeze.)

**Expiration date for use:**　1 year from the shipping date

**[Required Reagent, Apparatus and Equipment]**

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

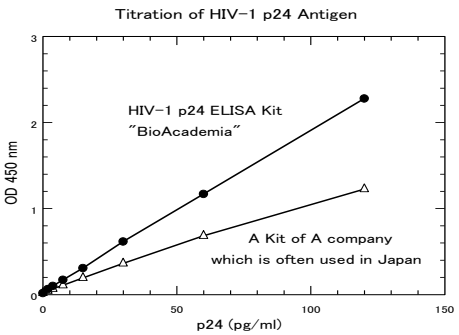



**Figure　Standard curve of HIV-1 p24　measurement by the 2 hour assay.**

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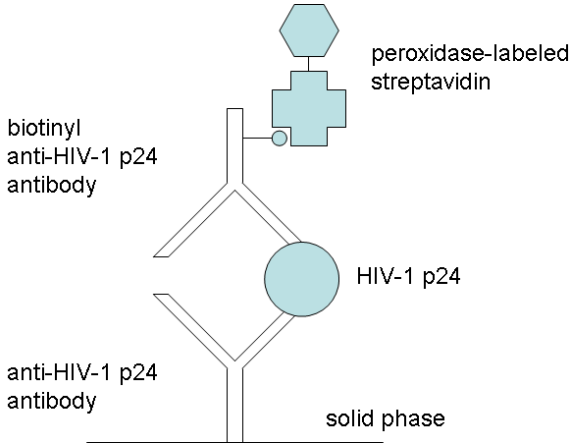
# HIV-1 p24 ELISA Kit "BioAcademia"
# Instruction Manual

**[Usage]**

This kit can measure the amounts of HIV-1 Gag p24 antigen in cell culture medium and of the lentivirus vectors derived from HIV-1 handily by a sandwich ELISA (Enzyme Linked Immunosorbent Assay) method. Since p24 antigen is a structure protein of HIV-1, the virus amounts in the sample can be measured from titration of p24 with this kit.

**[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/$H_2O_2$ | 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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Storage： 2 – 8℃ (Please don't freeze.)

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**[Required Reagent, Apparatus and Equipment]**

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3. Micropipettes and tips
4. Microplate reader

**[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 µl of 20-fold diluted Wash Buffer twice.
2) Add 200 µl of the diluted Antigen Standard or specimen solutions into the washed wells, and incubate at 37℃* for 2 hours.
3) Remove the solutions in the wells by aspiration, and wash the wells with 350 µl of 20-fold diluted Wash Buffer three times**.

Step 3

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

Step 4

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

Step 5

1) Add 100 µl of Substrate Solution into the washed wells, and incubate at room temperature for 30 minutes.
2) Add 100 µl 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℃ 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* "Safety factors involved in the extraction of biologically active proteins from human immunodeficiency virus." *J Virol Methods* **70**: 113-115 (1998) PMID: 9506820

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