Institut für HIV Forschung
SOP #21-00 **(April 2015)**

Perkin Elmer p24 ELISA Kit Protocol

Reagents

All items from the PerkinElmer HIV-1 P24 ELISA kit (5 96-well plates).
Catalogue No. NEK050B
Note: There are several items included in the kit that are not used in this protocol.

	Item	Amount used per plate
1	**5% Triton X-100**	100 ul per sample
2	**96-well microplate** (coated with monoclonal Ab. to HIV-1 p24)	1 plate
3	**Positive control**, 200ng/ml	10 ul
4	**Detector Ab**. (Rabbit polyclonal anti-p24 antibody in PBS)	11 ml
5	**Streptavidin-HRP Concentrate** (100-fold concentrate)	110 ul
6	**Streptavidin-HRP Diluent** (PBS with BSA and 0.05% Tween-20)	11 ml
7	**Substrate Diluent** (Citrate buffer with 0.03% hydrogen peroxide)	11 ml
8	**OPD Tablets**	1 tablet
9	**Stop Solution** (4N sulfuric acid)	11 ml
10	**Plate Wash Concentrate**, 20X (phosphate buffer and 1% Tween-20)	~200 ml
11	**Plate covers**	4 covers

Sample Preparation and Dilution

To harvest and inactivate sample supernatant, add 1 ml of supernatant to 100 ul of 5% Triton X-100 before removing it from the hood. Now the sample can be diluted on the bench. Note: If you are working with smaller volumes of supernatant, a similar dilution can be made. (i.e. 500 ul of sample with 50 ul of 5% Triton X-100.)

Because the kit only measures p24 levels between 0-400 pg/ml, most samples expected to be higher than that range will need to be diluted before adding to the assay. Dilute samples in R10 (RPMI with 10% FCS) in a 96-well plate, using a multichannel pipette to do multiple samples at once.

1:10 dilution - 270 ul R10 + 30 ul sample
1:100 dilution – 270 ul R10 + 30 ul 1:10 dilution
1:1000 dilution – 270 ul R10 + 30 ul 1:100 dilution

etc…

Standard Curve

The concentration of the positive control is 200 ng/ml. Use the following dilutions to create the 8-well standard curve. One set of dilutions will be enough for 2 plates.

Tube 1: 490 ul R10 + 10 ul (200 ng/ml) positive control = 4 ng/ml
Tube 2: 900 ul R10 + 100 ul Tube 1 = 400 pg/ml
Tube 3: 500 ul R10 + 500 ul Tube 2 = 200 pg/ml
Tube 4: 500 ul R10 + 500 ul Tube 3 = 100 pg/ml
Tube 5: 500 ul R10 + 500 ul Tube 4 = 50 pg/ml
Tube 6: 500 ul R10 + 500 ul Tube 5 = 25 pg/ml
Tube 7: 500 ul R10 + 500 ul Tube 6 = 12.5 pg/ml
Tube 8: 500 ul R10 + 500 ul Tube 7 = 6.25 pg/ml
Tube 9: 500 ul R10 = 0.0 pg/ml (blank)

Load 200 ul of Tubes 2-9 on the far right column of the plate as the standard curve.
DO NOT ADD TUBE 1 TO PLATE.

Sample Loading

- Each kit comes with five plates. Each plate contains 12 8-well strips that fit into a plastic frame.
- Take out any strips you do not need for your experiment and save for later use.
- Example: If you only have four samples to test and need 4 dilutions for each, 4 x 4 = 16 wells, plus 8 wells for the standard curve. You will only need 3 strips. 9 strips can be saved for later.
- Use tape to secure the edges of the strips to the plate, so that they will not fall out during later washes.
- Load 200ul of each sample into the wells. Do not let pipe tip touch the bottom of the plate. (The plates are coated with an antibody to capture p24).
- Cover the plate with enclosed cover slips and incubate at 37° for 2 hours.

Prepare Wash Buffer

- Dilute 1 bottle of 20x plate wash solution (100 ml) in 1900 ml single distilled water in a large flask.
- Fill squirt bottles with 1x solution for later wash steps.

Wash Step

- After 2 hour incubation, wash plate with 1x wash buffer.
- Remove the cover slip, dump the liquid forcefully into the sink so that you don't contaminate other wells.
- Wash the wells 6 times with the squirt bottle.
- Blot the plate on a blue pad before adding the next reagent.

Detector Antibody

- Measure out Detector Antibody based on the number of strips you are using, 100 ul per well (a good rule of thumb is 1 ml per strip giving you a little extra)
- Pour Detector Antibody into a clean reservoir and load using a multichannel pipette.
- Cover and incubate the plate at 37° for 1 hour.

Repeat Wash Step

Streptavidin-HRP

- Add 10 ul of Streptavidin-HRP concentrate for every 1 ml of "Streptavidin-HRP Diluent" (1:100 dilution).
- Mix the solution by inverting the conical several times.
- Pour the solution into a clean reservoir and add 100 ul per well.
- Cover and incubate the plate at room temperature in the dark for 30 minutes. (Cover in foil)

Repeat Wash Step

OPD Substrate

- Add 1 OPD tablet to 11 ml of "Substrate Diluent." Invert conical to mix and allow tablet to dissolve. Cover conical in foil, because of light sensitivity.
- Pour OPD solution into a clean reservoir and add 100 ul per well.
- Cover and incubate the plate at room temperature in the dark for 15-20 minutes.
- Due to toxicity, dispose of the OPD waste into a marked bottle and dispose through the chemical office.

Stop Solution

- Once standard curve has sufficiently changed color, add "Stop Solution" (sulfuric acid!) to halt the color-changing reaction.
- Pour Stop Solution into a clean reservoir and add 100 ul per well.

Read Plate

- Read the ELISA plate using the Vmax reader and Softmax software.
- Choose the p24 template to read the plate correctly, at wavelengths between 490-650.
- The program will generate a graph of the standard curve and calculated values.
- Manually input standard curve values and dilution factors for each well on the plate.
- Dispose of plate waste (OPD and stop solution 1:1) into a separate waste bottle (using a vacuum and collection flask) and dispose of it through the chemical office.

Protocol Summary

Step #: Reagent	Volume per Well	Incubation Time	Other Conditions
1: Samples	200 ul	2 hours	37°
2: Detector Ab	100 ul	1 hour	37°
3: Strep-HRP	100 ul (1:100 dilut)	30 minutes	Room temp, dark
4: OPD	100 ul (1 tablet/11 ml)	15-20 minutes	Room temp, dark
5: Stop Solution	100 ul	Read immediately	