


STANDARD OPERATING PROCEDURE			
	Perkin Elmer p24 ELISA Kit Protocol		SOP# TC-SOP-012
	Originated by:	Alicja Trocha	Date: 25 December 17
	Reviewed By:	Amruta Samant	Pages: 1 of 4
	Approved by:	Alicja Trocha	

I. PURPOSE

The purpose of this procedure is to establish and outline the requirements for attire and personal protective equipment (PPE) for Ragon Institute laboratories.

II. SCOPE

This procedure applies to all employees, students, contractors and visitors that enter and/or work in the Ragon Institute laboratories.

III. RESPONSIBILITIES

- A. The Ragon Institute **Director and all Laboratory Principal Investigators** are responsible for the overall implementation of this procedure and ensuring compliance.
- B. **Environmental Health & Safety (EH&S)** is responsible for periodically reviewing the application and maintenance of this procedure, and initiating any updates to this procedure.
- C. **All employees, students, contractors and visitors** are required to follow this procedure. Non-compliance with this procedure will result in the assignment of a corrective action plan.
- D. **The Partners Institutional Biosafety Committee, Ragon Laboratory Managers and/or EH&S** are responsible for outlining additional PPE beyond what is stated in Section IV. (Procedure) if warranted through a regulatory requirement or industry best-practice.

IV. PROCEDURE


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

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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.

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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, $4 \times 4 = 16$ 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.

A. 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)

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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	