revvity

HIV-1 P24 ELISA

Catalog Numbers
NEK050 One 96-well plate
NEK050A Two 96-well plates
NEK050B Five 96-well plates

Limitations of Use

For Research Use Only

Not for use in Diagnostic Procedures

Do Not Use Beyond Expiration Date

CAUTION: Revvity provides HIV-1 P24 ELISA products for research purposes only. In assuming receipt of this product, you acknowledge your obligation to use the product in accordance with the stated limitations of use.

TABLE OF CONTENTS

I. PROPRIETARY NAME

Revvity Health Sciences B.V., HIV-1 p24 ELISA.
Catalog Numbers: NEK050001KT, NEK050A001KT, NEK050B001KT

II. USAGE

This 'Research Use Only' kit (RUO) is designed to detect HIV-1 p24 core antigen (HIV-1 p24) in human serum or plasma or in cell culture supernatant.
Limitations of Use: For Research Use Only. Not for Use in Diagnostic Procedures. By accepting receipt of this product, you acknowledge your obligation to use the product in accordance with the stated limitations of use.
Limitations of Use within the European Union:
The Alliance HIV-1 P24 ELISA kit contains Triton X-100.
Triton X-100 is identified as a substance of environmental concern in the European Union and is added to the REACH Annex XIV Authorisation List. REACH Regulation provides an exemption for scientific research and development, defined as any scientific experimentation, analysis or chemical research carried out under controlled conditions in a volume less than 1 tonne per year (Article 3(23)).
Use of the Alliance HIV-1 P24 ELISA kit for scientific research and development with proper waste disposal according to local regulations is covered under this exemption.
By accepting receipt of this product, you acknowledge your obligation to use the product in accordance with the stated limitations of use.

III. BACKGROUND INFORMATION

Acquired Immune Deficiency Syndrome (AIDS) and a variety of related disorders are associated with infection by a human retrovirus, known as human immunodeficiency virus, type 1 (HIV-1). The Revvity HIV-1 p24 ELISA is an enzyme immunoassay for the detection of HIV-1 p24 antigen.

A 24 kilodalton protein (p24), immunologically distinct from proteins in most other retroviruses, has been demonstrated to be a major structural core component of HIV-1. The preparation of a mouse monoclonal antibody with high specificity and affinity for this viral protein has allowed the development of an enzyme-linked immunosorbent assay (ELISA) for HIV-1 p24.

During HIV-1 infection, antibodies are produced to viral antigens. These specific antibodies then bind to viral antigens and form immune complexes. Bound antigen is no longer detectable by antigen capture ElISAs. The Revvity HIV-1 p24 ELISA kit provides reagents for the disruption of antigen/antibody complexes allowing the previously bound antigen to be measured in serum and plasma samples.

IV. PRINCIPLES OF THE PROCEDURE

The Revvity kit provides reagents for immune complex disruption (ICD) of antigen/antibody complexes in serum and plasma samples using a combination of low pH and heat. The samples are then neutralized and transferred to microplate wells which are coated with a highly specific mouse monoclonal antibody to HIV-1 p24. The immobilized monoclonal antibody captures both free HIV-1 p24 and that which has been released upon disruption of immune complexes in the serum/plasma sample. Cell culture samples do not require disruption and are added directly to the monoclonal antibody-coated microplate wells. The captured antigen is complexed with biotinylated polyclonal antibody to HIV-1 p24, followed by a streptavidin-HRP (horseradish peroxidase) conjugate. The resulting complex is detected by incubation with ortho-phenylenediamine-HCl (OPD) which produces a yellow color that is directly proportional to the amount of HIV-1 p24 captured. The absorbance of each microplate well is determined using a microplate reader and calibrated against the absorbance of an HIV-1 p24 antigen standard or standard curve. Samples with absorbance values equal to or greater than the cutoff factor are considered initially reactive, but should be retested in duplicate to determine whether the reactivity is reproducible.

V. REAGENTS AND EQUIPMENT

A. Kit Components

Reagents are supplied for one, two, or five 96-well microplate(s).

1. **Antibody-coated Microplate** — One (2), (5) 96-well microplate(s) coated with monoclonal antibody to HIV-1 p24. Preservative: 0.01% Proclin-300.

2. **Positive Control, 200 ng/mL** — One (1), (2) tube(s), 0.4 mL/tube. Contains 200 ng/mL as HIV-1 p24 (approx. 800 ng/mL as total HIV-1 protein), in PBS plus BSA and Triton X-100. Preservative: < 0.1% sodium azide.

3. **Negative Control** — One (2), (5) bottle(s), 13 mL/bottle. Recalcified human serum. Preservative: 0.5% 2-chloroacetamide. Non-reactive for Hepatitis B surface antigen and antibodies to HIV-1, HIV-2, and HCV.

4. **Detector Antibody** — One (2), (5) bottle(s), 11 mL/bottle. Rabbit polyclonal anti-p24 antibody in PBS containing animal sera, casein and human serum non-reactive for Hepatitis B surface antigen and antibodies to HIV-1, HIV-2, and HCV. Preservative: 0.2% Proclin-300 and < 0.1% Sodium Azide.

5. **Streptavidin-HRP Concentrate** — One (2), (4) vial(s), 0.4 mL/vial. A 100-fold concentrate of Streptavidin-HRP in citrate buffer with BSA and detergent. Preservative: 0.5% 2-chloroacetamide.

6. **Streptavidin-HRP Diluent** — One (2), (5) bottle(s), 14 mL/bottle. PBS with BSA and 0.05% Tween-20. Preservative: 0.5% 2-chloroacetamide.

7. **Glycine Reagent** — One (1), (2) bottle(s), 25 mL/bottle, 1.5M Glycine.

8. **Tris Reagent** — One (1), (2) bottle(s), 25 mL/bottle, 1.5M Tris.

9. **Substrate Diluent** — One (1), (2) bottle(s), 60 mL/bottle. Citrate buffer containing 0.03% hydrogen peroxide. Stabilizer: 0.002% sodium stannate.

10. **Immune Complex Control** — One (1), (1) bottle, 1.5 mL/bottle. Human serum plus HIV-1 p24 antibody and inactivated HIV-1 p24 antigen. Non-reactive for Hepatitis B surface antigen and antibodies to HCV and HIV-2. Preservative: < 0.5% 2-chloroacetamide.

11. **5% Triton X-100** — One (1), (2) bottle(s), 6 mL/bottle. 5% Triton X-100 in phosphate buffer plus an inert blue dye. Preservative: 0.02% sodium azide.

12. **OPD Tablets** — One (1), (2) strip(s), 5 tablets/strip. Foil-wrapped OPD tablets.

13. **Stop Solution** — One (2), (5) bottle(s), 12 mL/bottle. 4N sulfuric acid.

14. **Plate Wash Concentrate, 20X** — Two (3), (5) bottles, 100 mL/bottle. Concentrated phosphate buffer plus 1% Tween-20. Preservative: 2% 2-chloroacetamide.

15. **Plate Covers** — Twelve (24), (36).

B. Storage of Kit Components

Stop Solution, Plate Wash Concentrate, 20X, and **Plate Covers** may be stored at room temperature (15–30°C). All other kit components should be kept refrigerated at 2–8°C.

C. Stability of Kit Components

Changes in the physical appearance of the reagents supplied may indicate instability or deterioration of these materials. Do not use reagents which are visibly turbid.

OPD substrate solution should be colorless or very pale yellow. A yellow-orange color indicates deterioration and the solution must NOT be used. The substrate solution should be prepared within 15 minutes of use and protected from light.

D. Additional Equipment and Reagents Required

1. Uncoated microplates. Uncoated microplates can be obtained from Revvity (Cat.# 6055640).

2. Precision pipettors plus tips-

-Multichannel pipettor with volume capacity to 200 µL

-Single channel pipettors to deliver 10–1000 µL.

3. Vortex mixer.

4. Polypropylene tubes.

5. Disposable gloves.

6. Disposable reagent reservoirs.

7. Automated plate washer OR syringe-multichannel port manifold apparatus for manual plate wash dispensing.

8. Pump and vacuum dome or aspirator flask if needed for automated washer. A double trap system is recommended.

9. Incubator capable of maintaining 37 ± 1°C.

10. Microplate reader with 490 or 492 nm and > 600 nm filter capability. Follow installation, operation, calibration and maintenance instructions provided by manufacturer.

VI. WARNINGS AND PRECAUTIONS

A. Safety Considerations

Limitations of Use: For Research Use Only. Not for Use in Diagnostic Procedures. In assuming receipt of this product, you acknowledge your obligation to use the product in accordance with the stated limitations of use.

1. Each donor unit of human sera or plasma used in the preparation of this product was tested by FDA licensed methods for the presence of antibodies to HIV-1, HIV-2, HCV, and for hepatitis B surface antigen and found to be negative (not repeatably reactive). The **Positive Control, 200 ng/mL** has been inactivated by psoralen/UV irradiation and

detergent treatment. The **Immune Complex Control** has been inactivated by Beta propiolactone/UV irradiation. However, because no known method can offer full assurance that infectious agents are absent or have been completely inactivated, these components must be handled using good laboratory practice to avoid skin contact and ingestion.

2. Do not pipette by mouth.

3. Wear disposable gloves throughout the test procedure. Wear suitable protective clothing and eye/face protection when handling chemical hazards. Dispose of gloves in the biohazard waste. Thoroughly wash hands afterwards.

4. Wipe non-acid containing spills promptly with 1% sodium hypochlorite (1:5 dilution of liquid household bleach in water). Spills involving acids should be collected into absorbent towels and the dried spill area wiped with 1% sodium hypochlorite. Contaminated materials should be disposed of in the biohazard waste.

5. Dispose of all materials and samples used in the biohazard waste. The recommended method of disposal is autoclaving for a minimum of one hour at 121°C. Disposable materials may be incinerated. Mix liquid wastes with an equal volume of 5%sodium hypochlorite allowing for at least 60 minutes for disinfection.

6. Do not allow **OPD Tablets** to come into contact with metal or other oxidizing agents. If skin is contacted, flush with water. Solutions containing OPD should be disposed of in compliance with local regulations.

7. Sodium azide (NaN_3) is used in **5% Triton X-100, Positive Control, 200 ng/mL** and **Detector Antibody** as a preservative and may react with lead or copper in drain lines to form explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide buildup in drains.

B. Performance Considerations

1. Do not use reagents beyond the kit expiration date.

2. Use only the reagent lots assigned to the kit. Do not interchange vials or bottle caps and stoppers.

3. Addition of reagents must be in the order specified. Reagents and samples must be added to the plate in a timely manner.

4. After completion of each wash step, samples or the next reagent should be added promptly. DO NOT ALLOW PLATE TO DRY AFTER WASHING.

5 Plate washing may be automated, semi-automated or manual, but MUST BE CARRIED OUT WITH CARE to ensure optimal performance of the assay. It is recommended that six remove-fill cycles be performed as below:

- Automatic Microplate Washer — Use two 3-cycle washes of at least 300 µL diluted (1X) wash buffer per well per wash. After each 3-cycle wash, blot the plate by inverting and firmly tapping it on absorbent paper. Also, reorient the plate in the washer between cycles by turning it 180 degrees (if applicable).

- Manual Microplate Washer — Wash six (6) times, using 300 µL diluted (1X) wash buffer per well per wash. Fill the entire plate, then aspirate in the same order. Blot the plate after the third and the last wash.

- Hand-held Syringe or Squirt Bottle — Wash six (6) times, using 300 µL diluted (1X) wash buffer per well per wash. Blot the plate after each wash.

VII. SAMPLE COLLECTION, PROCESSING AND STORAGE

The Revvity HIV-1 p24 ELISA kit may be used with human serum or plasma or cell culture supernatants. The effects of storage on the detectability of HIV-1 antigens is unknown. Minimize the time thawed samples remain unfrozen prior to assay. If samples are to be stored, they should be frozen at -20°C or below and multiple freezethaws should be avoided. Do not use a self-defrosting freezer.
Serum and plasma samples should be processed on the same day as collected. If samples are not assayed on the day of collection, they should be stored frozen at -20°C or below until tested. Clear, non-hemolyzed specimens should be used whenever possible.

VIII. ASSAY PROCEDURE

SERUM/PLASMA ICD FORMAT

NOTE: Cell Culture supernatants and non-ICD format serum/plasma samples do not undergo immune complex disruption. See instructions for these samples in section VIII.2., page 15.

A. Reagent Preparation

1. Equilibrate all reagents to room temperature (15–30°C) prior to use.

2. Dilute **Plate Wash Concentrate, 20X** to 1X by adding one part plate wash concentrate to 19 parts distilled, deionized water. Crystals may form in the **Plate Wash Concentrate, 20X** if refrigerated. These should be redissolved by gentle warming prior to use. Approximately 1000 mL of diluted (1X) wash buffer is needed per plate assayed. More or less may be needed depending on the type of washer used. Diluted (1X) wash buffer should be prepared fresh prior to assay.

   Prepare all other working reagents within 15 minutes of use. Prepare only enough for the assay being run. Discard any excess.

B. Immune Complex Disruption (ICD) of Serum/Plasma Samples

3. Determine the number of uncoated microplate strips needed. Each plate or partial plate must include one substrate blank, three **Negative Control,** two 400 pg/mL diluted **Positive Control** and two **Immune Complex Control** wells. All controls and samples must be acid disrupted and neutralized.

4. **Preparation of Positive Control Working Concentration** (400 pg/mL):

Dilute the **Positive Control, 200 ng/mL** with **Negative Control** to the 400 pg/mL working concentration:

p24 Conc.(pg/mL)	Tube Label	NEGATIVE CONTROL (µL)	ADDITION (µL)
4000	A	980	20 POS CTRL
400	B	900	100 Tube A

Tube B at the working concentration of 400 pg/mL will be used for addition to the plate.

NOTE: If a standard curve is to be run, follow quantitative assay instructions as outlined in Appendix I.

5. Add 20 µL **5% Triton X-100** to all uncoated microplate wells except substrate blank.

6. Add 90 µL of **Negative Control** serum, 400 pg/mL diluted **Positive Control** (tube B), **Immune Complex Control,** and samples to designated uncoated microplate wells.

7. Add 90 µL **Glycine Reagent** to strip 1 of the uncoated microplate using a multichannel pipettor. Gently mix well contents by slowly drawing up and dispensing the contents five times. Change the pipettor tips. Continue to add 90 µL **Glycine Reagent** to all wells, one strip at a time, ensuring

adequate mixing and changing of pipettor tips between each strip.

8. Seal plate and incubate for 60 ± 5 minutes at 37 ± 1°C.

C. Neutralization

9. Add 90 µL **Tris Reagent** to strip 1 using a multichannel pipettor and mix five times. Continue adding 90 µL **Tris Reagent** to all wells, one strip at a time, ensuring adequate mixing and changing of pipettor tips between each row.

10. Incubate plate ten to twenty minutes at room temperature (15–30°C).

D. Control/Sample Transfer and Incubation

11. During the neutralization period, remove the **Antibody-coated Microplate** from its sealed pouch. Use same number of strips as used in Step B.3. Return excess strips to supplied bag containing desiccant, seal, and store at 2–8°C.

12. Using a multichannel pipettor, mix contents of strip 1 of the uncoated microplate several times. Transfer 150 µL to strip 1 of the **Antibody-coated Microplate.** Change the pipettor tips. Continue mixing, transferring and changing tips as for strip 1 until all controls and samples have been transferred to the **Antibody-coated Microplate.**

13. Seal plate and incubate two hours ± 5 minutes at 37 ± 1°C.

E. Detector Antibody

14. Wash plate six times with diluted (1X) wash buffer. Plate washing may be automated, semi-automated or manual but must be carried out with care to

ensure optimal assay performance. Six wash cycles of at least 300 µL/well with diluted (1X) wash buffer are recommended. (See page 10, Section VI.B.5., Performance Considerations, for detailed wash instructions.) Blot well before addition of next reagent.

15. Add 100 µL **Detector Antibody** to all wells except substrate blank.

16. Seal plate and incubate 60 ± 5 minutes at 37 ± 1°C.

F. Streptavidin-HRP (SA-HRP)

17. Wash plate as described in step 14. Blot well.

18. Within 15 minutes of use, dilute sufficient **Streptavidin-HRP Concentrate** to the 1:100 working concentration with **Streptavidin-HRP Diluent.** Mix thoroughly.

SA-HRP 1:100 WORKING DILUTION:

Number of strips	SA-HRP (mL)	SA-HRP DILUENT (mL)
4	0.040	4.0
6	0.060	6.0
8	0.080	8.0
12	0.120	12.0
24	0.220	22.0

19. Add 100 µL diluted SA-HRP to all wells except substrate blank.

20. Seal plate and incubate 30 ± 5 minutes at room temperature (15–30°C).

G. OPD Substrate Solution

21. Wash plate as described in step 14. Blot well.

22. Prepare sufficient OPD Substrate Solution within 15 minutes of use. With non-metallic forceps or the equivalent, add one **OPD Tablet** to 11 mL of **Substrate Diluent** for each plate or partial plate assayed. Vortex vigorously to assure complete dissolution. Protect from light. The OPD substrate solution should be colorless to pale yellow. A yellow-orange color indicates that the reagent is contaminated and must be discarded.

23. Add 100 µL OPD substrate solution to all wells including substrate blank.

24. Seal plate and incubate 30 ± 5 minutes at room temperature (15–30°C) in the dark.

H. Stop/Read Plate

25. Stop the reaction by adding 100 µL of **Stop Solution** to all wells.

26. Read the plate at 490 or 492 nm, blanking the plate reader on air. (Consult plate reader Instruction Manual for specific directions for instrument blanking.) Readings must be taken with a reference filter at > 600 nm. The plate should be read within 15 minutes after stopping the reaction. Be sure the bottom of the plate is clean and dry prior to reading.

NON-ICD FORMAT FOR CELL CULTURE SUPERNATANT OR SERUM/PLASMA

A. Reagent Preparation

1. Equilibrate all reagents to room temperature (15–30°C) before use.

2. Dilute **Plate Wash Concentrate, 20X** to 1X by adding one part plate wash concentrate to 19 parts distilled, deionized water. Crystals may form in the **Plate Wash Concentrate, 20X** if refrigerated. These should be redissolved by gentle warming prior to use. Approximately 1000 mL of diluted (1X) wash buffer is needed per plate assayed. More or less may be needed depending on the type of washer used. Diluted (1X) wash buffer should be prepared fresh prior to assay.

   Prepare all other working reagents within 15 minutes of use. Prepare only enough for the assay being run. Discard any excess.

B. Control and Sample Incubation

3. Determine the number of **Antibody-Coated Microplate** strips needed for assay. Each plate or partial plate should include one substrate blank, three negative controls, and two 100 pg/mL diluted **Positive Control** wells.

4. **Preparation of Positive Control Working Concentration** (100 pg/mL)

   Dilute **Positive Control, 200 ng/mL** to the 100 pg/mL working concentration. Use uninoculated cell culture media as the diluent for assays of culture supernatants. **Negative Control** should be used as the diluent for assays of serum or plasma samples:

Standard Conc. (pg/mL)	Tube Label	DILUENT (µL)	ADDITION (µL)
4000	A	980	20 POS CTRL
100	B	975	25 Tube A

Tube B at the working concentration of 100 pg/mL will be used for addition to the plate.

NOTE: If a standard curve is to be run, follow quantitative assay instructions as outlined in Appendix I.

5. Add 20 µL **Triton X-100** to all wells except substrate blank.

6. Add 200 µL of the appropriate diluent (**Negative Control** or uninnoculated cell culture media) to the three wells designated as for negative control. Add 200 µL of the 100 pg/mL diluted **Positive Control** (tube B) and samples to designated wells. Mix well with pipettor.

7. Seal plate and incubate for two hours at 37 ± 1°C.

C. Detector Antibody

8. Wash plate six times with diluted (1X) wash buffer. Plate washing may be automated, semi-automated or manual but must be carried out with care to ensure optimal assay performance. Six wash cycles of at least 300 µL/well with diluted (1X) wash buffer are recommended. (See page 10, Section VI. B.5., Performance Considerations, for detailed wash instructions.) Blot well before addition of next reagent.

9. Add 100 µL **Detector Antibody** to all wells except substrate blank.

10. Seal plate and incubate 60 ± 5 minutes at 37 ± 1 °C.

D. Streptavidin-HRP (SA-HRP)

11. Wash plate as described in step 8. Blot well.

12. Within 15 minutes of use, dilute sufficient **Streptavidin-HRP Concentrate** to the 1:100 working concentration with **Streptavidin-HRP Diluent.** Mix thoroughly

SA-HRP 1:100 WORKING DILUTION

Number of strips	SA-HRP (mL)	SA-HRP DILUENT (mL)
4	0.040	4.0
6	0.060	6.0
8	0.080	8.0
12	0.120	12.0
24	0.220	22.0

13. Add 100 µL diluted SA-HRP to all wells except substrate blank.

14. Seal plate and incubate 30 ± 5 minutes at room temperature (15–30°C).

E. OPD Substrate Solution

15. Wash plate as described in step 8. Blot well.

16. Prepare sufficient OPD Substrate Solution within 15 minutes of use. With non-metallic forceps or the equivalent, add one **OPD Tablet** to 11 mL of **Substrate Diluent** for each plate or partial plate assayed. Vortex vigorously to assure complete dissolution. Protect from light. The OPD substrate solution should be colorless to pale yellow. A yellow-orange color indicates that the reagent is contaminated and must be discarded.

17. Add 100 µL OPD substrate solution to all wells including substrate blank.

18. Seal plate and incubate 30 ± 5 minutes at room temperature (15–30°C) in the dark.

F. Stop/Read Plate

19. Stop the reaction by adding 100 µL of **Stop Solution** to all wells.

20. Read the plate at 490 or 492 nm, blanking the plate reader on air. (Consult plate reader Instruction Manual for specific directions for instrument blanking.) Readings must be taken with a reference filter at > 600 nm. The plate should be read within 15 minutes after stopping the reaction. Be sure the bottom of the plate is clean and dry prior to reading.

IX. CALCULATIONS

The following abbreviations are used in the following sections. All represent the O.D. value of a well:

SB = Substrate Blank
NC = Negative Control
PC = Diluted Positive Control

A. Plate Acceptability Criteria

1. SB < 0.050

2. NC < 0.150 for at least two of the three wells and for calculation of the mean NC.

3. PC individual well > 0.600 and PC mean > 0.800.

4. The **Immune Complex Control** should be reactive; in the quantitative assay, it should have a value of approximately 100 pg/mL.

If any one of these acceptability criteria are <u>not</u> met, the plate (or partial plate) is considered to be invalid. <u>All</u> samples tested on the invalid plate or partial plate must be repeated.

B. Calculation of Sample Reactivity

l. Calculate the **Cutoff** for each plate or partial plate.

Add 0.050 to the mean absorbance (O.D.) of the NC wells.

Example:

NCs	0.024, 0.020, 0.022
Mean NC	0.022

Cutoff = 0.022 + 0.050 = 0.072

2.

Sample O.D. < Cutoff	**Not Initially Reactive (NIR)**
Sample O.D. ≥ Cutoff	**Initially Reactive (IR)**

3. Reactive samples should be tested again in duplicate.

Calculate the Cutoff as in A.

Both Sample Wells < Cutoff	**Not Repeat Reactive (NRR)**
One or Both Sample Wells ≥ Cutoff	**Repeat Reactive (RR)**

X. LIMITATIONS OF PROCEDURE

A. Cross-Reactivity

1. The following materials have been checked and found to exhibit no detectable cross-reactivity:

Uninfected CD4 + Cell Lines: H9, Molt3a, Molt4. Uninfected Monocyte Lines: U-937, MonoA 3.5, MonoA 4.5. Uninfected mixed PBL Cultures

Azidothymidine, 0.5 mM; Dideoxycytidine, 0.5 mM; Ribavirin, 0.5 mM; HPA-23, 0.5 mM; Foscarnet, 5 mM.
Similarly, no cross-reactivity was detected with culture fluid from 2 herpes simplex virus isolates, 2 cytomegalovirus isolates and 5 Epstein-Barr virus isolates.

2. Reactivity was found in the following HIV isolates: 3B, RF, Z84, Z34, AL and MN.

XI. PERFORMANCE CHARACTERISTICS

SERUM/PLASMA ICD FORMAT

A. Recovery

A recovery study was done by adding a known quantity (200 pg/mL) of HIV-1 p24 to six different serum pools.

Serum Pool	p24 added (pg/mL)	p24 measured (pg/mL)	% Recovery
1	200	175.2	87.6
2	200	185.4	92.7
3	200	179.1	89.6
4	200	182.3	91.2
5	200	184.4	92.2
6	200	189.3	94.7

Mean Recovery 91.3%

B. Reproducibility

Precision was determined by multiple duplicate analyses of several HIV-1 p24 antigen positive serum/plasma samples. Each sample was tested in duplicate in four runs of two plates each by two operators.

Sample	n	Mean Value (pg/mL)	Within Assay CV (%)	Between Assay CV (%)
A	32	20.8	2.9	22.7
B	32	31.9	3.9	18.0
C	32	200.9	2.1	13.2

C. Linearity

HIV-1 p24 was added to six different serum pools and diluted 1:2, 1:4, and 1:8. Results are reported in pg/mL, corrected for dilution factor.

Serum Pool	Dilution Factor			
	1:1	1:2	1:4	1:8
1	346.0	350.4	341.0	325.7
2	436.6	370.8	380.7	328.4
3	347.9	358.2	351.9	401.5
4	349.4	364.6	346.4	333.8
5	370.0	368.8	332.8	309.5
6	362.6	378.6	360.2	339.2

D. Analytical Sensitivity

1. HIV-1 p24 was added to six different serum pools. Serial dilutions were made of each using the matching individual serum as the diluent. Analytical sensitivity was determined as the lowest concentration of HIV-1 p 24 which was reactive in all six pools tested.

   Analytical sensitivity: 26 pg/mL

2. Alternatively, analytical sensitivity was determined via least squares fit to the standard curve at an absorbance equal to the cutoff (i.e., mean negative control O.D. + 0.050).

Analytical sensitivity (least squares fit): 17.1 pg/mL

NON-ICD FORMAT FOR SERUM/PLASMA

A. Recovery

A recovery study was done by adding a known quantity (50 pg/mL) of HIV-1 p24 to six different serum pools.

Serum Pool	p24 added (pg/mL)	p24 measured (pg/mL)	% Recovery
1	50	37.0	74.1
2	50	50.4	100/8
3	50	48.7	97.5
4	50	64.8	129.5
5	50	54.1	108.1
6	50	53.7	107.3

Mean Recovery: 102.9%

B. Reproducibility

Precision was determined by multiple duplicate analyses of several HIV-1 antigen-positive serum/plasma samples. Each sample was tested in duplicate in four runs of two plates each by two operators.

Sample	n	Mean Value pg/mL	Within Assay CV (%)	Between Assay CV (%)
A	32	5.5	6.3	18.2
B	32	6.7	5.7	10.0
C	32	19.1	4.9	7.0
D	32	54.6	5.1	3.6

C. Linearity

HIV-1 p24 was added to six different serum pools and diluted from 1:2 to about 1:10. Results are reported in pg/mL, corrected for the dilution factor.

Serum Pool	Dilution Factor				
	1:1	1:2	1:3.91	1:7.63	1:9.52
1	78.1	74.1	78.7	70.2	67.8
2	97.4	100.8	104.3	103.1	93.4
3	98.4	97.5	92.8	87.3	84.6
4	101.6	129.5	118.1	98.4	93.4
5	111.2	108.1	100.9	116.1	108.9
6	98.2	107.3	88.9	78.4	81.0

D. Analytical Sensitivity

1. HIV-1 p24 was added to six different serum pools. Serial dilutions were made of each using the matching individual serum as the diluent. Analytical sensitivity was determined as the lowest concentration of HIV-1 p24 which was positive in all six pools tested.

   Analytical sensitivity: 4.3 pg/mL

2. Alternatively, analytical sensitivity was determined via the least squares fit to the standard curve at an absorbance equal to the cutoff (i.e., mean negative control O.D. + 0.050).

Analytical sensitivity (least squares fit): 3.5 pg/mL

NON-ICD FORMAT FOR CELL CULTURE SUPERNATANT

A. Recovery

Six cell culture supernatants, several of which were already reactive for HIV-1 p24, were spiked with an additional 25 pg/mL p24. Recovery was determined as the % expected value, the latter being the sum of the initial sample p24 quantity plus 25 pg/mL.

Culture Sample	Initial Value (pg/mL)	Spiked Value (pg/mL)	Total Expected Value (pg/mL)	% Expected
1	*NR	20.2	25.0	80.9
2	NR	21.4	25.0	85.4
3	NR	33.1	25.0	132.3
4	NR	27.5	25.0	110.0
5	4.2	34.0	29.2	116.6
6	53.5	75.7	78.5	96.4

*NR = Non-Reactive (i.e., sample O.D. < cutoff O.D.)

Mean Recovery: 103.6%

XII. REFERENCES

1. Allain, J.P., Laurian, Y., Paul, D.A., Verroust, F., Leuther, M., Gazengel, C., Senn, D., Larrieu, M.J. and Bosser, C. Long-term evaluation of HIV antigen and antibodies to p24 and gp41 in patients with hemophilia. Potential clinical importance. N. Engl. J. Med. 317:1114-1121 (1987).

2. Barre-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. and Montagnier, L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220: 868-871 (1983).

3. Biggar, R.J., Melbye, M., Ebbesen, P., Alexander, S., Nielsen, J.0., Sarin, P. and Faber, V. Variation in human T lymphotropic virus III (HTLV-III) antibodies in homosexual men: decline before onset of illness related to acquired immune deficiency syndrome (AIDS). Br. Med. J. 291:997-998 (1985).

4. Casey, J.M., Kim, Y., Andersen, P.R., Watson, K.F., Fox, J.L. and Devare, S.G. Human T-cell lympphotropic virus type III: immunologic characterization and primary structure analysis of the major internal protein, p24. J. Virol. 55:417-423 (1985).

5. Forster, S.M., Osborne, L.M., Cheingsong-Popov, R., Kenney, C., Burnell, R., Jeffries, D.J., Pinching, A.J., Harris, J.R. and Weber, J.N. Decline of anti-p24 antibody precedes antigenaemia as correlate of prognosis in HIV-1 infection. AIDS 1:235-240 (1987).

6. Goudsmit, J., Lange, J.M., Paul, D.A. and Dawson, G.J. Antigenemia and antibody titers to core and envelope antigens in AIDS, AIDS-related complex, and subclinical human immunodeficiency virus infection. J. Infect. Dis. 155:558-560 (1987).

7. Higgins, J.R., Pedersen, N.C. and Carlson, J.R. Detection and differentiation by sandwich enzyme-linked immunosorbent assay of human T-cell lymphotropic virus type III/lymphadenopathy-associated virus- and acquired

immunodeficiency syndrome-associated retroviruslike clinical isolates. J. Clin. Microbiol. 24:424-430 (1986).

8. Hoffman, A.D., Banapour, B. and Levy, J.A. Characterization of the AIDS-associated retrovirus reverse transcriptase and optimal conditions for its detection in virions. Virology 147:326-335 (1985).

9. Homsy, J., Luciw, P., Cheng-Mayer, C. and Levy, J., Abstract, Int. Congress on AIDS, Paris, June 23 - 25, 1986.

10. Kalyanaraman, V.S., Sarngadharan, M.G., Poiesz, B., Ruscetti, F.W. and Gallo, R.C. Immunological properties of a type C retrovirus isolated from cultured human T-lymphoma cells and comparison to other mammalian retroviruses. J. Virology 38:906-915 (1981).

11. Lange, J.M., Coutinho, R.A., Krone, W.J., Verdonck. L.F., Danner, S.A., Noordaa, J. and Goudsmit, J. Distinct IgG recognition patterns during progression of subclinical and clinical infection with lymphadenopathy associated virus/human T lymphotropic virus. Br. Med. J. 292:228-230 (1986).

12. Levy, J. A.,, Hoffman, A.D., Kramer, S.M., Landis, J.A., Shimabukuro, J.M., and Oshiro, L.S. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science 225:840-842 (1984).

13. McDougal, J.S., Cort, S. P., Kennedy, M.S., Cabridilla, C.D., Feorino, P.M., Francis, D.P., Hicks, D., Kalyanaraman, V.S. and Martin, L.S. Immunoassay for the detection and quantitation of infectious human retrovirus, lymphadenopathy-associated virus (LAV). J. Immunol. Methods 76:171-183 (1985).

14. Pan, L.Z., Cheng-Mayer, C. and Levy, J.A. Patterns of antibody response in individuals infected with the human immunodeficiency virus. J. Infect. Dis. 155:626-632 (1987).

15. Pedersen, C., Nielsen, C.M., Vestergaard, B.F., Gerstoft, J., Krogsgaard, K. and Nielsen, J.0. Temporal relation of antigenaemia and loss of antibodies to core antigens to development of clinical disease in HIV infection. Br. Med. J. 295:567-569 (1987).

16. Popovic, M., Sarngadharan, M.G., Read, E. and Gallo, R.C. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 224:497-500 (1984).

17. Sarngadharan, M.G., Bruch, L., Popovic, M. and Gallo, R.C. Immunological properties of the Gag protein p24 of the acquired immunodeficiency syndrome retrovirus (human Tcell leukemia virus type III). Proc. Natl. Acad. Sci. (USA) 82:3481-3484 (1985).

18. Spira, T.J., Kaplan, J.E., Feorino, P.M., Warfield, D.T., Fishbein, D.B. and Bozeman, L.H. Human immunodeficiency virus viremia as a prognostic indicator in homosexual men with lymphadenopathy syndrome. N. Engl. J. Med. 317:1093-1094 (1987).

19. Weber, J.N., Clapham, P.R., Weiss, R.A.,Parker, D., Roberts, C., Duncan, J., Weller, I., Carne, C., Tedder, R.S., Pinching, A. J., et al. Human immunodeficiency virus infection in two cohorts of homosexual men: neutralising sera and association of anti-gag antibody with prognosis. Lancet i:119-121 (1987).

20. Wittek, A.E., Phelan, M.A., Wells, M.A., Vujcic, L.K., Epstein, J.S., Lane, H.C. and Quinnan, G.V. Jr. Detection of human immunodeficiency virus core protein in plasma by enzyme immunoassay. Association of antigenemia with symptomatic disease and T-helper cell depletion. Ann. Intern. Med. 107:286-292 (1987).

21. Wong-Staal, F. and Gallo, R.C. Human T-lymphotropic retroviruses. Nature 317:395-403 (1985).

22. Levy, J.A. Pathogenesis of human immunodeficiency virus infection. Microbiol. Rev. 57:183-289 (1993).

23. Nishanian, P., Huskins, K.R., Stehn, S., Detels, R., and Fahey, J.L. A simple method for improved assay demonstrates that HIV p24 antigen is present as immune complexes in most sera from HIV-infected individuals. J. Infect. Dis. 162:21-28 (1990).

24. Bollinger, R.C. Jr., Kline, R.L., Francis, H.L., Moss, M.W., Bartlett, J.G., and Quinn, T.C. Acid dissociation increases the sensitivity of p24 antigen detection for the evaluation of antiviral therapy and disease progression in asymptomatic human immunodeficiency virus-infected persons. J. Infect. Dis. 165:913-916 (1992).

XIII. NAME AND PLACE OF MANUFACTURE

Revvity Health Sciences B.V.
Rigaweg 22
9723TH Groningen
The Netherlands
Toll Free: (800) 762-4000 or
(+1) 203-925-4602
www.revvity.com

XIV. APPENDIX I: QUANTITATIVE ASSAY

SERUM/PLASMA ICD FORMAT

A. Preparation of Standard Curve

Prepare standard curve by diluting the **Positive Control, 200 ng/mL**, using the **Negative Control** serum as the diluent.

STANDARD (pg/mL	TUBE LABEL	DILUENT (µL)	ADD (µL)
4000	A	980	20 POS. CONT.
400	B	900	100 Tube A
200	C	500	500 Tube B
100	D	500	500 Tube C
50	E	500	500 Tube D
25	F	500	500 Tube E

Tubes B-F (25–400 pg/mL) should be used as the standard curve for ICD assays.

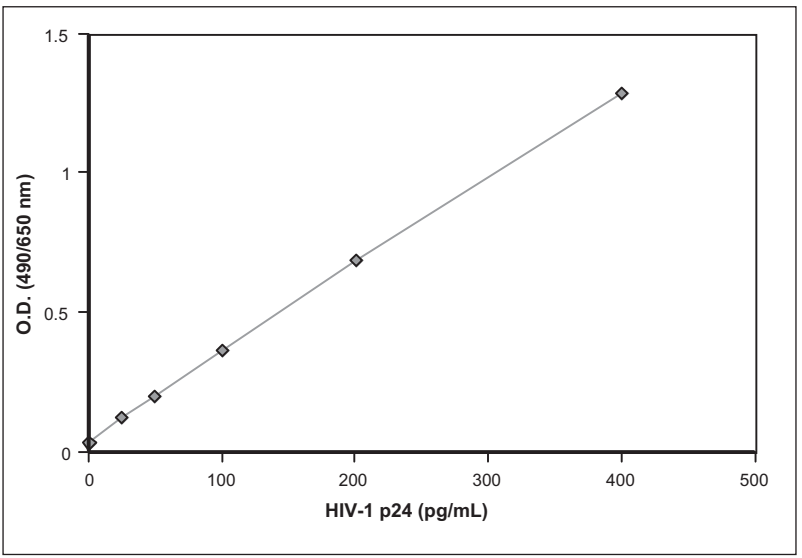
B. Sample Calculations

1. Determine sample reactivity by comparing sample O.D. to **Cutoff**. (See Section IX. Calculations).

2. Plot mean O.D.s for each standard (y-axis) versus the concentration of HIV-1 p24 (x-axis) using graph paper or quadratic regression.

3. Determine the concentration of HIV-1 p24 for each reactive sample by interpolation from the standard curve.

C. Typical Standard Curve



HIV-1 p24 Standard Concentration pg/mL	Mean O.D. 490/650 nm
0	0.034
25	0.126
50	0.200
100	0.367
200	0.690
400	1.287

NON-ICD FORMAT FOR CELL CULTURE SUPERNATANT OR SERUM/PLASMA

A. Preparation of Standard Curve

Prepare standard curve by diluting the Positive Control, 200 ng/mL, using uninnoculated cell culture media as a diluent for cell culture samples. Use Negative Control serum as the diluent for serum/plasma samples.

STANDARD (pg/mL)	TUBE LABEL	DILUENT (µL)	ADD (µL)
4000	A	980	20 Pos. Cont.
100	B	975	25 Tube A
50	C	500	500 Tube B
25	D	500	500 Tube C
12.5	E	500	500 Tube D

Tubes B-E (12.5–100 pg/mL) should be used as the standard curve for cell culture and non-ICD serum/plasma assays.

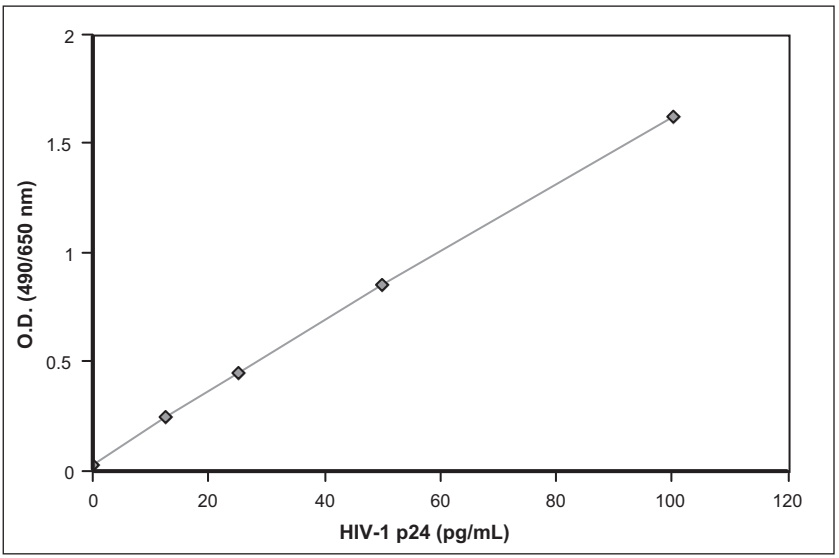
B. Sample Calculations

1. Determine sample reactivity by comparing sample O.D. to Cutoff. (See Section IX. Calculations).

2. Plot mean O.D.s for each standard (y-axis) versus the concentration of HIV-1 p24 (x-axis) using graph paper or quadratic regression.

3. Determine the concentration of HIV-1 p24 for each reactive sample by interpolation from the standard curve.

C. Typical Standard Curve



HIV-1 p24 Standard Concentration pg/mL	Mean O.D. 490/650 nm
0	0.027
12.5	0.247
25	0.452
50	0.852
100	1.625

MANUFACTURED BY:

Revvity Health Sciences B.V.
Rigaweg 22
9723TH Groningen
The Netherlands
Toll Free: (800) 762-4000 or
(+1) 203-925-4602
www.revvity.com

NOTES

Worldwide Headquarters:
Revvity Inc.
940 Winter Street
Waltham, Massachusetts 02451 USA
Phone: (800) 762-4000 or (+1) 203-925-4602

For any support, please visit www.revvity/contact-us

EXPP00040 - 10/23 - REV. E

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
Tel. (800) 762-4000
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com