NEN™ Life Science Products

HIV-1 p24 CONFIRMATORY REAGENTS

Catalog Number NEK059

For Research Use Only

Caution: A Research Chemical for Research Purposes Only.

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I. PROPRIETARY NAME

NEN™ Life Science Products HIV-1 p24 Confirmatory Reagents.
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II. INTENDED USE

These reagents are designed to confirm the presence of HIV-1 p24 antigen in samples found to be repeatably reactive by the NEN HIV-1 p24 ELISA.

III. PRINCIPLES OF THE PROCEDURE

The HIV-1 p24 ELISA is a sandwich immunoassay designed to detect the presence of HIV-1 p24 in human serum or plasma and cell culture supernatants. Samples found to be repeatably reactive in the NEN HIV-1 p24 ELISA should be tested with the NEN HIV-1 p24 Confirmatory Reagents. These reagents are suitable for confirming the presence of HIV-1 p24 antigen detected in either the Immune Complex Disruption (ICD) or non-ICD format of the NEN HIV-1 p24 ELISA.

A repeatably reactive response generated in the HIV-1 p24 ELISA is confirmed by neutralization of specific signal upon incubation of the sample with antibody to HIV-1 p24 (in the form of human anti-HIV). This incubation forces free HIV-1 p24 antigen in the sample into immune complexes which will not be captured by the anti-HIV-1 p24 coated microplate wells upon subsequent re-assay.

Samples to be confirmed are prepared in duplicate. Confirmatory Reagent (human antibody to HIV) is added to one duplicate. Control Reagent (human antibody nonreactive for HIV-1) is added to the other duplicate. Both samples are then processed with the HIV-1 p24 ELISA kit using either the ICD or non-ICD format. A reduction of signal by at least 50% (between the duplicates) in the presence of the Confirmatory Reagent is confirmation of a positive HIV-1 p24 viral antigen result.

IV. REAGENTS AND EQUIPMENT

Sufficient **Confirmatory** and **Control Reagents** are provided to perform 50 confirmation tests in the ICD format or 100 confirmation tests in the non-ICD format (including controls).

A. Kit Components

1. **Confirmatory (Neutralizing) Reagent** - One vial, 1.0 mL/vial. Human serum positive for antibodies to HIV. Non-reactive for Hepatitis B surface antigen and antibodies to HCV. Preservative: 0.5% 2-chloroacetamide.

2. **Control Reagent** - One vial, 1.0 mL/vial. Human serum nonreactive for antibodies to HIV-1, HIV-2, HCV, and Hepatitis B surface antigen. Preservative: 0.5% 2-chloroacetamide.

B. Storage of Kit Components

Store Confirmatory Reagents at 2-8°C. When stored under these conditions, the reagents are stable until the labeled expiration date.

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.

D. Equipment and Reagents Required

In addition to the reagents supplied with the kit, the following materials are required:

1. Uncoated 96-well microplates. Uncoated microplates can be obtained from NEN (NEK073; 5 microplates /per package).

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. Syringe-multichannel port manifold apparatus for manual plate wash dispensing OR automated plate washer.

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

V. WARNINGS AND PRECAUTIONS

A. Safety Considerations

1. CAUTION: HUMAN SOURCE MATERIAL. HANDLE AS POTENTIALLY INFECTIOUS.

Each donor unit of human sera or plasma used in the preparation of the Control Reagent was tested by FDA licensed methods for the presence of antibodies to HIV-1, HIV-2, and HCV as well as for Hepatitis B surface antigen and found to be negative (not repeatably reactive).

The Confirmatory Reagent contains human serum positive for antibodies to HIV, and 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 reagents 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. 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 specimens 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.

B. Performance considerations

1. Do not use reagents beyond the 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.

VI. SAMPLE COLLECTION, PROCESSING AND STORAGE

The NEN HIV-1 p24 Confirmatory kit may be used with human serum or plasma and 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 freeze-thaws 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.

VII. ASSAY PROCEDURE

Samples found repeatably reactive in either of the NEN HIV-1 p24 ELISA formats (ICD or non-ICD) should be confirmed. In the ICD format, **Control** and **Confirmatory Reagents** are added to samples after acid disruption. In the non-ICD format, **Control and Confirmatory Reagents** are added to samples as the first step. Confirmatory assays are then processed according to the instructions in the NEN HIV-1 p24 ELISA kit.

A. ICD CONFIRMATORY FORMAT FOR SERUM/PLASMA

1. Reagent Preparation:

a. Equilibrate all reagents to room temperature (15-30°C) before use.

b. 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 must be redissolved by gentle warming prior to use. Approximately 1000 mL of diluted wash buffer is needed per plate processed. 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.

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

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

b. **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.

c. Add 20 µL of **5% Triton X-100** (from the NEN HIV-1 p24 ELISA kit) to all uncoated microplate wells except the substrate blank.

d. Add 90 µL of **Negative Control**, 400 pg/mL diluted **Positive Control** (from Tube B), **ICC**, and samples to the designated uncoated microplate wells.

e. Add 90 µL of **Glycine Reagent** to strip 1 of the uncoated microplate with a multichannel pipettor. Mix the reagents in the wells by slowly drawing up and dispensing the contents 5 times. Change the pipettor tips. Continue to add 90 µL **Glycine Reagent** to all wells, one strip at a time, followed by mixing and changing tips between each row.

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

3. Addition of Confirmatory and Control Reagents:

a. Add 20 µL **Control Reagent** to the three **Negative Control** wells, two of the four 400 pg/mL diluted **Positive Control** wells, and to one each of the **ICC** and sample wells.

b. Add 20 µL **Confirmatory Reagent** to the remaining 400 pg/mL diluted **Positive Control**, **ICC** and sample wells.

4. Neutralization:

a. Immediately after addition of appropriate Confirmatory Reagents, add 90 µL **Tris Reagent** to strip 1 using a multichannel pipettor and mix five times. Change the pipettor tips. 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.

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

5. To complete the assay, follow instructions in the NEN HIV-1 p24 ELISA kit for the Serum/plasma ICD format, Section VIII.1., beginning with Step D.11 Control/Sample Transfer and Incubation.

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

1. Reagent Preparation:

a. Equilibrate all reagents to room temperature (15-30°C) before use.

b. 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 must be redissolved by gentle warming prior to use. Approximately 1000 mL of diluted (1X) wash buffer is needed per plate processed. 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.

2. Addition of Confirmatory and Control Reagents:

a. Determine the number of uncoated microplate strips needed for the assay. (Alternatively, polypropylene tubes may be used to carry out these steps). Each plate or partial plate must include one substrate blank well, three negative control wells and four 100 pg/mL diluted **Positive Control** wells. Each sample to be confirmed must be added to the uncoated microplate in duplicate.

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

Dilute the **Positive Control, 200 ng/mL**, to the 100 pg/mL working concentration using **Negative Control** as the diluent when testing serum/plasma samples. Uninoculated cell culture media must be used as the diluent when testing cell culture supernatants.

p24 Conc (pg/mL)	Tube Label	DILUENT (µL)	ADDITION (µL)
4000	A	980	20 POS CTRL
100	B	1170	30 Tube A

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

c. Add 25 µL of **5% Triton X-100** (from the NEN HIV-1 p24 ELISA kit) to all wells of the uncoated microplate.

d. Add 225 µL of the appropriate diluent (**Negative Control** or uninoculated cell culture media) to the three wells designated for the negative control, 100 pg/mL diluted **Positive Control** (from Tube B), and samples to appropriate uncoated microplate wells.

e. Add 10 µL **Control Reagent** to each of three negative control wells, two of the four 100 pg/mL **Positive Control** wells and one each of the duplicate sample wells. Add 10 µL **Confirmatory Reagent** to the remaining 100 pg/mL diluted **Positive Control** and sample wells.

f. Using a multichannel pipettor, mix contents of strip 1 on the uncoated plate 5 times. Change the pipettor tips and continue mixing each strip with a new set of tips until all strips have been mixed.

g. Seal plate and incubate for 20-30 minutes at 37 ± 1°C.

3. Transfer and Incubation of Controls and Samples:

a. During the incubation period, remove the **Antibody-coated microplate** from the NEN HIV-1 p24 ELISA kit. Use the same number of strips as used in Step B.3. above. Seal excess strips in supplied bag containing desiccant and store at 2-8°C.

b. Using a multichannel pipettor, mix contents of strip 1 on the uncoated microplate several times. Transfer 200 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**.

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

d. **To complete the assay**, **follow instructions in NEN HIV-1 p24 ELISA kit, Non-ICD Format for Cell Culture Supernatant or Serum/Plasma, Section VIII. 2., beginning with Step C.8.: Detector Antibody.**

VIII. CALCULATIONS

The following abbreviations are used in subsequent specified criteria. Each represents the O.D. value of a well:

SB = Substrate Blank

NC = Negative Control with Control Reagent

PC = Diluted Positive Control with Control Reagent

A. Plate Acceptability Criteria

1. SB $\leq$ 0.050

2. NC $\leq$ 0.150 for at least two of the three wells and for calculation of the mean NC.

3. PC individual wells = 0.600 and PC mean $\geq$ 0.800.

4. % Reduction PC $\geq$ 50%.

   Calculate the % reduction in O.D. of the diluted Positive Control (PC).

   $$\% \text{ Reduction PC} = \frac{(PC - PCN)}{(PC - NC)} \times 100$$

   PC = Mean O.D. for Positive Control with Control Reagent

   NC = Mean O.D. for Negative Control with Control Reagent

   PCN = mean O.D. for Positive Control with Confirmatory Reagent

   The % reduction in O.D. must be greater than 50% for the assay to be valid.

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. Determination of Sample Reactivity

1. Calculate the **Cutoff** for each plate or partial plate. Add 0.040 to the mean NC with Control Reagent O.D.

   Example:

NC (with Control Reagent) O.D.'s: 0.024, 0.020, 0.022

Mean NC (with Control Reagent) O.D.: 0.022

Cutoff = 0.022 + 0.040 = 0.062

2. Determine Sample Reactivity:

Sample + Control
Reagent O.D. < Cutoff Non Reactive

Sample + Control
Reagent O.D. ≥ Cutoff Reactive

3. Calculate % reduction in O.D. for each Reactive sample in the Confirmatory Assay.

$$\% \text{ Reduction Sample} = \frac{(S - SN)}{(S - NC)} \times 100$$

S = O.D. for Sample with Control Reagent

NC = Mean O.D. for Negative Control with Control Reagent.

SN = O.D. for Sample with Confirmatory Reagent

4. a. A sample is **Confirmed Reactive (CR)** for HIV-1 p24 when

The O.D. of the sample containing the Control Reagent is greater than or equal to the cutoff AND the percent reduction in O.D. of the sample containing the confirmatory reagent is greater than or equal to 50%.

b. A sample is considered **Not Confirmed Reactive (NCR)** for HIV-1 p24 when:

The O.D. of the sample containing the Control Reagent is less than the cutoff OR the O.D. of the sample well containing the Control Reagent is above the cutoff AND the percent reduction in O.D. of the sample containing the Confirmatory Reagent is less than 50%.

c. A sample may be considered **Indeterminate** when the O.D,. of the sample containing the Control Reagent is 2.0 or above AND the percent reduction in O.D. of the sample containing the Confirmatory Reagent is less than 50%. Such samples should be diluted 1:20 or greater and re-tested in the Confirmatory Assay.

IX. NAME AND PLACE OF MANUFACTURE

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