



HTRF HIV P24 DETECTION KITS

PROTOCOL

Part # 64P24PEG & 64P24PEH

Test size#: 500 tests (64P24PEG) and 10,000 tests (64P24PEH) - assay volume: 20 μ L

Revision: 02 - April 2022

Store at: -60°C or below (64P24PEG); -60°C or below (64P24PEH)

For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of HIV p24 in supernatant and offers a fast alternative to ELISA.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, HIV p24 is detected in a sandwich assay by using anti HIV p24 antibody labeled with Europium cryptate (donor), and anti HIV p24 antibody labeled with d2 (acceptor).

When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the HIV p24 concentration.

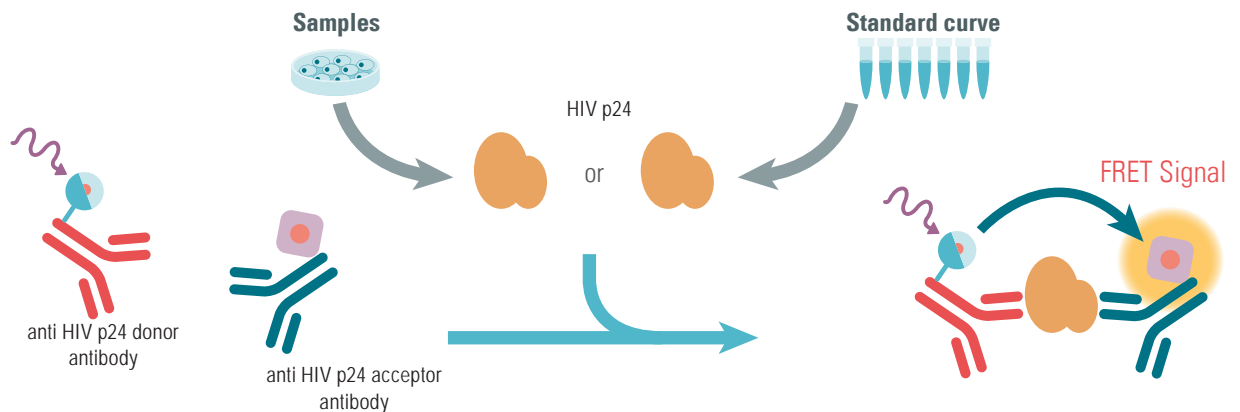
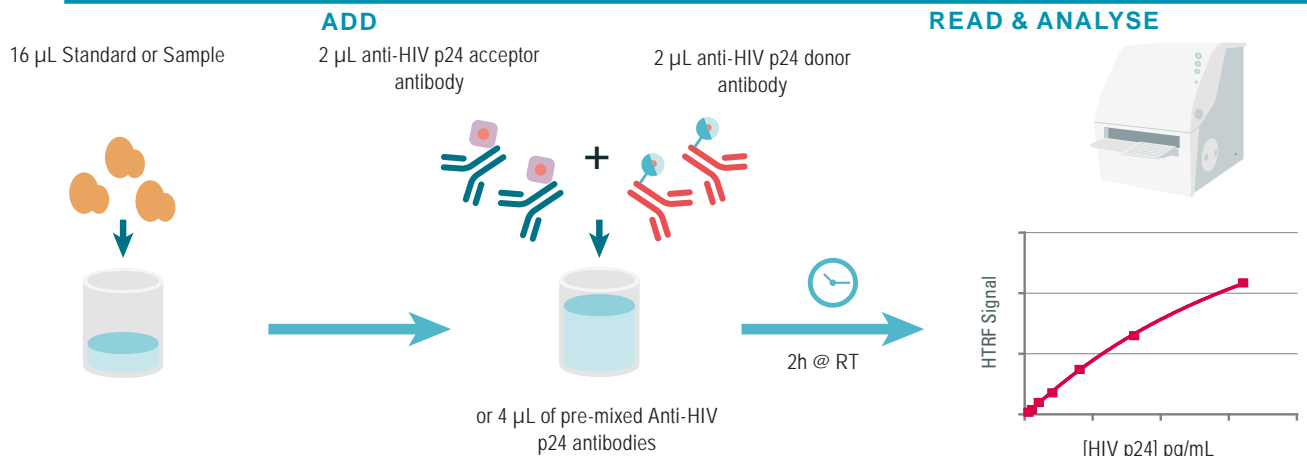


Figure 1: Principle of HTRF HIV p24 sandwich assay.

PROTOCOL AT A GLANCE



Make sure to use the set-up for Eu Cryptate.

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS * CAT # 64P24PEG	10,000 TESTS * CAT # 64P24PEH
HIV p24 Standard Frozen	1 vial - 50 μ L 200 ng/mL	1 vial - 50 μ L 200 ng/mL
HIV p24 Eu Cryptate Antibody	1 vial - 20 μ L Frozen - 50X	1 vial - 0.4 mL Frozen - 50X
HIV p24 d2 Antibody	1 vial - 20 μ L Frozen - 50X	1 vial - 0.4 mL Frozen - 50X
Diluent #5 ** 5X	1 vial 2 mL	1 vial 130 mL
Detection buffer *** ready-to-use	2 vials 1.5 mL Detection Buffer #3	1 vial 50 mL Detection Buffer #3

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 μ L final volume..

Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.

** Medium like cell culture medium can be an alternative to the diluent.

*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

PURCHASE SEPARATELY:

- HTRF®-Certified Reader. **Make sure the setup for Eu Cryptate is used.**

For a list of HTRF-compatible readers and set-up recommendations, please visit www.cisbio.com/compatible-readers

- Small volume (SV) detection microplates. For information about microplate recommendations, please visit our website at: cisbio.com/microplates-recommendations

STORAGE AND STABILITY

Store the kit at -60°C or below.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label. Diluent and detection buffer are shipped frozen, but can be stored at $2-8^{\circ}\text{C}$ in your premises.



Reagents

If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below .






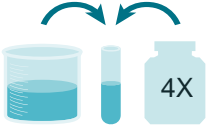
Volume of HIV p24 standard aliquots should not be under 10 μ L.

REAGENT PREPARATION**BEFORE YOU BEGIN:**

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature, allow them to warm up to room temperature for at least 30 mins before use
- Before use, allow Diluent and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- HIV p24 standards (for standard curve) must be prepared in diluent or in the same medium as the samples.

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

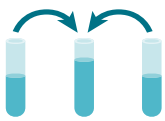
TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64P24PEG			10,000 TESTS KIT - 64P24PEH
Anti-HIV p24 Eu Cryptate antibody			
Thaw the HIV p24 Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.			Thaw the HIV p24 Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.
Anti-HIV p24 d2 antibody			
Thaw the HIV p24 d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.			Thaw the HIV p24 d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.
HIV p24 Standard			
Thaw the HIV p241 standard stock solution (200 ng/mL) at RT. Mix gently. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solution into disposable plastic vials for storage at -60°C or below.			Thaw the HIV p241 standard stock solution (200 ng/mL) at RT. Mix gently. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solution into disposable plastic vials for storage at -60°C or below.
Diluent			
Dilute 5-fold the 5 X diluent #5 with distilled water: homogenize the 5 X diluent #5 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water (e.g., 1 mL of diluent + 4 mL of distilled water). Mix gently after dilution. This 1X diluent can be frozen and stored at -60°C or below.	4 vol		1 vol
Detection buffer			
The Detection buffer is ready-to-use.			The Detection buffer is ready-to-use.

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 2 µL of HIV p24-Eu Cryptate Antibody and 2 µL of HIV p24-d2 Antibody.

Prepare the two antibody solutions in separate vials.

500 TESTS KIT - 64P24PEG			10,000 TESTS KIT - 64P24PEH
HIV p24 Eu Cryptate antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of HIV p24 Eu Cryptate antibody with Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of detection buffer (e.g. 20 µL of Eu Cryptate antibody stock solution + 980 µL of detection buffer).	1 vol	49 vol	Dilute 50-fold the 50X stock solution (thawed reagent) of HIV p24 Eu Cryptate antibody with Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of detection buffer (e.g. 0.4 mL of Eu Cryptate antibody stock solution + 19.6 mL of detection buffer).
HIV p24 d2 antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of HIV p24 d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of detection buffer (e.g. 20 µL of d2-antibody stock solution + 980 µL of detection buffer).	1 vol	49 vol	Dilute 50-fold the 50X stock solution (thawed reagent) of HIV p24 d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of detection buffer (e.g. 0.4 mL of d2 antibody stock solution + 19.6 mL of detection buffer).
Antibody mix			
It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).			It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 20 mL of d2 antibody + 20 mL of Cryptate antibody).

TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 16 μL of standard.
- Dilute the standard stock solution serially with diluent #5 (1X) or in the medium used for the preparation of the samples.
- If culture medium is used to dilute the standard, we recommend to supplement it with serum (2 to 10%) or BSA (0.2 to 1%) in order to avoid HIV p24 sticking to assay plates.
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in diluent #5 (1X).
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

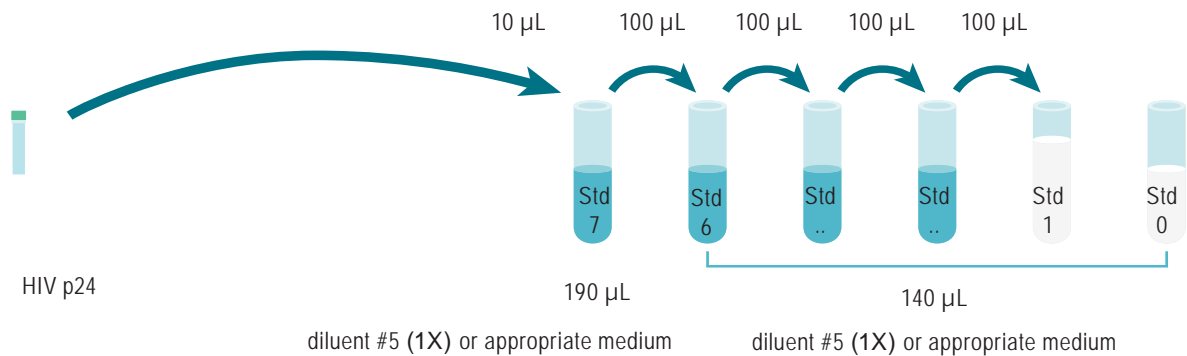
Dilute the standard stock solution 20-fold with diluent; this yields the Standard Max solution (10,000 pg/mL)

Dilute the standard stock solution 20-fold with diluent #5 (1X) to prepare high standard (Std 7): e.g. take 10 μL of standard stock solution and add it to 190 μL of diluent #5 (1X). Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/2.4 serial dilutions as follows:

- Dispense 140 μL of diluent #5 (1X) in each vial from Std 6 to Std 0.
- Add 100 μL of standard to 140 μL of diluent #5 (1X), mix gently and repeat the 1/2.4 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Negative control) is diluent #5 (1X) or appropriate culture medium alone.








STANDARD	SERIAL DILUTIONS	HIV p24 WORKING SOLUTIONS (pg/mL)
Standard Stock solution	Thawed stock solution	200 000
Standard 7	10 μL Standard stock Solution + 190 μL diluent 1X	10 000
Standard 6	100 μL standard 7 + 140 μL lysis buffer 1X	4 167
Standard 5	100 μL standard 6 + 140 μL lysis buffer 1X	1 736
Standard 4	100 μL standard 5 + 140 μL lysis buffer 1X	723
Standard 3	100 μL standard 4 + 140 μL lysis buffer 1X	301
Standard 2	100 μL standard 3 + 140 μL lysis buffer 1X	126
Standard 1	100 μL standard 2 + 140 μL lysis buffer 1X	52
Standard 0	140 μL lysis buffer 1X	-

TO PREPARE SAMPLES:

- Each well requires 16 μ L of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 7) must be diluted diluent #5 (1X) in your appropriate sample medium, prepared, as recommended above.
- To obtain additional information or support, please contact the HTRF technical support team at cisbio.com/contact-us

ASSAY PROTOCOL

	Standard (Std 0 - Std 7)	Samples
Step 1 	Dispense 16 μ L of each HIV p24 standard (Std 0 - Std 7) into each standard well	Dispense 16 μ L of each sample into each sample well
Step 2 	Add 2 μ L of HIV p24 d2 antibody working solution to all wells	
Step 3 	Add 2 μ L of HIV p24 Eu Cryptate antibody working solution to all wells	
Step 4 	Seal the plate and incubate 2h @ RT	
Step 5 	Remove the plate sealer and read on an HTRF® compatible reader	

DATA REDUCTION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit <http://www.cisbio.com/htrf-ratio-and-data-reduction>

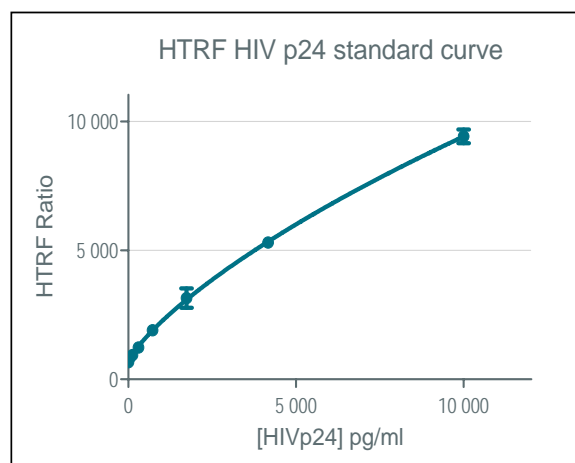
RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

Standard curve fitting with the 4 Parameter Logistic (4PL) model (with 1/Y² weighting):

	Ratio (1)	CV (2)
Standard 0 - Negative control	664	5%
Standard 1 - 52 pg/mL	837	7%
Standard 2 - 126 pg/mL	939	6%
Standard 3 - 301 pg/mL	1229	8%
Standard 4 - 723 pg/mL	1899	2%
Standard 5 - 1,736 pg/mL	3148	12%
Standard 6 - 4,167 pg/mL	5299	1%
Standard 7 - 10,000 pg/mL	9416	3%



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