MANUAL

Technology: HTRF® Biomarkers

HTRF AAV3B Capsid detection Kit

Part Numbers	64AAV3PEG	64AAV3PEH
Test Size	500 tests	10 000 tests

Storage: ≤-60°C or below

Version: 06 **Date:** March 2024

For research only. Not for use in diagnostic procedures

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Adeno-associated virus serotype 1 (AAV3B) particles (being expressed in Viral Particles/mL or VP/mL) in both cell lysates and supernatants and offers a fast alternative to ELISA.

AAVs are used as vector for gene delivery mainly because of their ability to infect cells without pathogenicity, and its ease of engineering and production. AAV3B is an efficient vector for skeletal muscle or central nervous system.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, AAV3B capsid is detected in a sandwich assay by using a pre-mixture of biotinylated anti-AAV3B antibody bound to the streptavidin Europium cryptate (donor), and a second pre-mixture of the HRP anti-AAV3B antibody bound to an anti-HRP 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 AAV3B capsid concentration.

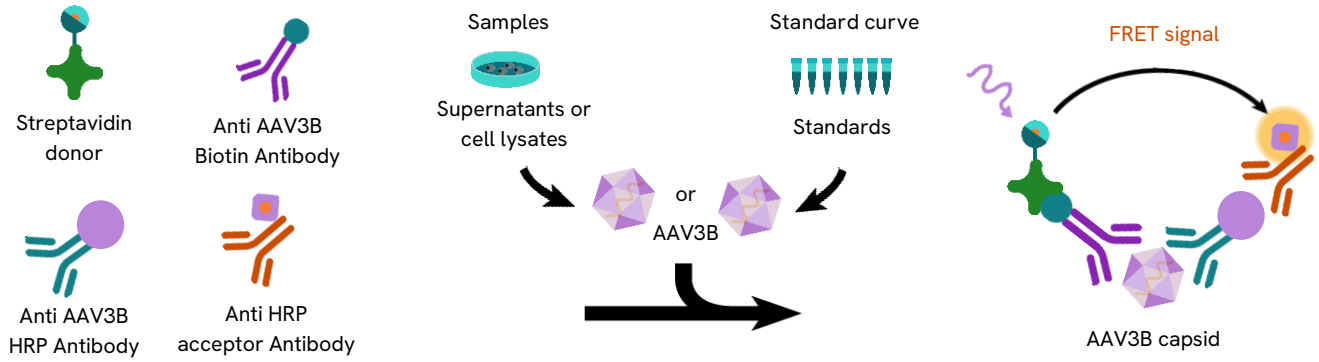
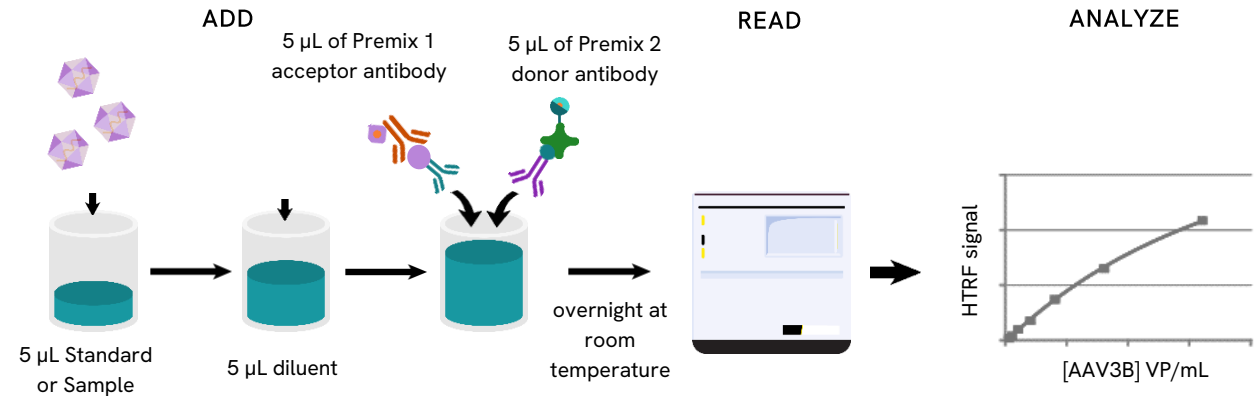


Figure 1: Principle of HTRF AAV3B sandwich assay.

PROTOCOL AT A GLANCE



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

MATERIAL PROVIDED

KIT COMPONENTS	500 TESTS*			10,000 TESTS*		
AAV3B Standard Frozen		green cap	1 vial 1.2E+12 VP/mL		green cap	2 vials 1.2E+12 VP/mL
Eu Cryptate Streptavidin Frozen 20X		orange cap	1 vial 65 µL		red cap	1 vial 1.25 mL
AAV3B Biotin Antibody Frozen 20X		orange cap	1 vial 65 µL		red cap	1 vial 1.25 mL
d2 Acceptor Antibody Frozen 20X		blue cap	1 vial 65 µL		purple cap	1 vial 1.25 mL
AAV3B HRP Antibody Frozen 20X		blue cap	1 vial 65 µL		purple cap	1 vial 1.25 mL
Diluent** #5 5X		yellow cap	1 vial 2 mL		white cap	1 vial 100 mL
Detection Buffer*** #3 Ready-to-use		transparent cap	1 vial 7 mL		red cap	1 vial 105 mL
Lysis Buffer **** #3 4X		transparent cap	1 vial 2 mL		transparent cap	1 vial 2 mL

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 µL 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.
**** The Lysis buffer is used to lysate or dilute samples.

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 our website
- Small volume (SV) detection microplates. For more information about microplate recommendations, please visit our website.

STORAGE AND STABILITY

Kit:

- 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°C in your premises.

Reagents:

- Once thawed, antibody and standard solutions can be frozen once.
- To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at ≤-60°C.
- Volume of standard and antibody aliquots should not be under 10 µL.
- Thawed diluent and detection buffer can be stored at 2-8°C in your premises.

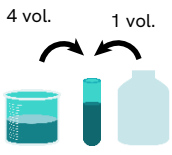
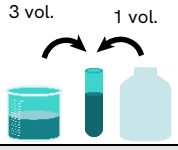
REAGENT PREPARATION

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.
- It is recommended to filter buffers.

- Antibody solutions and premix must be prepared in individual vials.
- AAV3B standards (for standard curve) must be prepared in diluent or in the same medium as the samples (cell culture medium or lysis buffer).

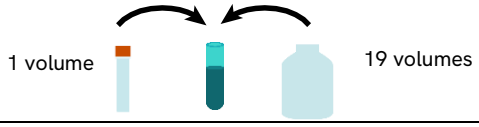
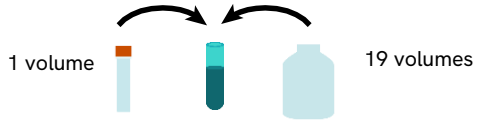
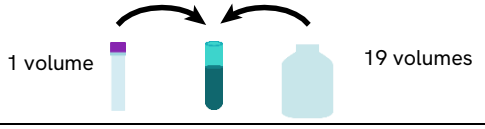
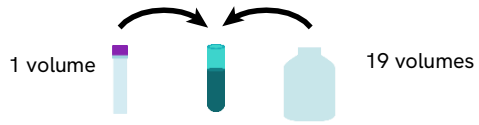
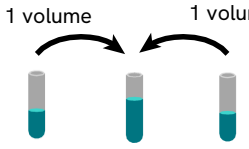
- **Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.**
- **To prepare reagent stock solutions:**

500 TESTS			10.000 TESTS
Eu Cryptate Streptavidin			
Thaw the Eu Cryptate Streptavidin. Mix gently. This 20X 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 Eu Cryptate Streptavidin. Mix gently. This 20X 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.
AAV3B Biotin antibody			
Thaw the AAV3B Biotin antibody. Mix gently. This 20X 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 AAV3B Biotin antibody. Mix gently. This 20X 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.
AAV3B HRP antibody			
Thaw the AAV3B HRP antibody. Mix gently. This 20X 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 AAV3B HRP antibody. Mix gently. This 20X 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.
d2 antibody			
Thaw the d2 antibody. Mix gently. This 20X 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 d2 antibody. Mix gently. This 20X 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.
AAV3B capsid Standard			
Thaw the AAV3B standard to obtain 1.2E+12 VP/mL stock solution. Mix gently. 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 AAV3B standard to obtain 1.2E+12 VP/mL stock solution. Mix gently. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions 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.			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., 20 mL of diluent + 80 mL of distilled water). Mix gently after dilution. This 1X diluent can be frozen and stored at -60°C or below.
Lysis buffer			
Dilute the “lysis buffer 4X” 4-fold with distilled water to prepare lysis buffer 1X. e.g. take 1.25 mL of lysis buffer 4X and add it to 3.75 mL of distilled water. Mix gently.			Dilute the “lysis buffer 4X” 4-fold with distilled water to prepare lysis buffer 1X. e.g. take 1.25 mL of lysis buffer 4X and add it to 3.75 mL of distilled water. Mix gently.
Detection buffer			

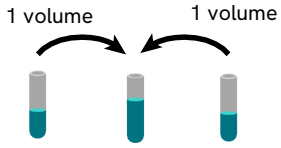
The Detection buffer is ready-to-use.

To prepare working solutions

Each well requires 5 µL of Premix 1 (AAV3B HRP antibody + d2 antibody) and 5 µL of Premix 2 (Eu Cryptate Streptavidin + AAV3B Biotin antibody). Prepare the antibody solutions in separate vials.

500 TESTS	10,000 TESTS
Eu Cryptate Streptavidin	
	
Dilute 20-fold the 20X stock solution (thawed reagent) of Eu Cryptate Streptavidin with Detection buffer #3: add 1 volume of Eu Cryptate Streptavidin stock solution in 19 volumes of detection buffer (e.g. 65 µL of Eu Cryptate Streptavidin stock solution + 1235 µL of detection buffer).	Dilute 20-fold the 20X stock solution (thawed reagent of Eu Cryptate Streptavidin with Detection buffer #3: add 1 volume of Eu Cryptate Streptavidin stock solution in 19 volumes of detection buffer (e.g. 1.25 mL of Eu Cryptate Streptavidin stock solution + 23.75 mL of detection buffer).
AAV3B Biotin antibody	
	
Dilute 20-fold the 20X stock solution (thawed reagent) of AAV3B Biotin antibody with Detection buffer #3: add 1 volume of AAV3B Biotin antibody stock solution in 19 volumes of detection buffer (e.g. 65 µL of AAV3B Biotin antibody stock solution + 1235 µL of detection buffer).	Dilute 20-fold the 20X stock solution (thawed reagent) of AAV3B Biotin antibody with Detection buffer #3: add 1 volume of AAV3B Biotin antibody stock solution in 19 volumes of detection buffer (e.g. 1.25 mL of AAV3B Biotin antibody stock solution + 23.75 mL of detection buffer).
AAV3B HRP antibody	
	
Dilute 20-fold the 20X stock solution (thawed reagent) of AAV3B HRP antibody with Detection buffer #3: add 1 volume of AAV3B HRP antibody stock solution in 19 volumes of detection buffer (e.g. 65 µL of AAV3B HRP antibody stock solution + 1235 µL of detection buffer).	Dilute 20-fold the 20X stock solution (thawed reagent) of AAV3B HRP antibody with Detection buffer #3: add 1 volume of AAV3B HRP antibody stock solution in 19 volumes of detection buffer (e.g. 1.25 mL of AAV3B HRP antibody stock solution + 23.75 mL of detection buffer).
d2 antibody	
	
Dilute 20-fold the 20X stock solution (thawed reagent) of d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 19 volumes of detection buffer (e.g. 65 µL of d2 antibody stock solution + 1235 µL of detection buffer).	Dilute 20-fold the 20X stock solution (thawed reagent) of d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 19 volumes of detection buffer (e.g. 1.25 mL of d2 antibody stock solution + 23.75 mL of detection buffer).
Premix 1 with AAV3B HRP antibody & d2 antibody	
Pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of AAV3B HRP antibody solution to 1 volume of d2 antibody solution (e.g. 1.3 mL of AAV3B HRP antibody + 1.3 mL of d2 antibody).	 Pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of AAV3B HRP antibody solution to 1 volume of d2 antibody solution (e.g. 25 mL of AAV3B HRP antibody + 25 mL of d2 antibody).
Premix 2 with Eu Cryptate Streptavidin & AAV3B Biotin antibody	

Pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of Eu Cryptate Streptavidin solution to 1 volume of AAV3B Biotin antibody solution (e.g. 1.3 mL of Eu Cryptate Streptavidin + 1.3 mL of AAV3B Biotin antibody).



Pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of Eu Cryptate Streptavidin solution to 1 volume of AAV3B Biotin antibody solution (e.g. 25 mL of Eu Cryptate Streptavidin + 25 mL of AAV3B Biotin antibody).

To prepare working standards solutions

- Each well requires 5 µL of standard.
- Dilute the standard stock solution serially with diluent #5 (1X) or with the cell culture medium or lysis buffer used to prepare your samples.
- 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 or lysis buffer and in diluent #5 (1X).
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

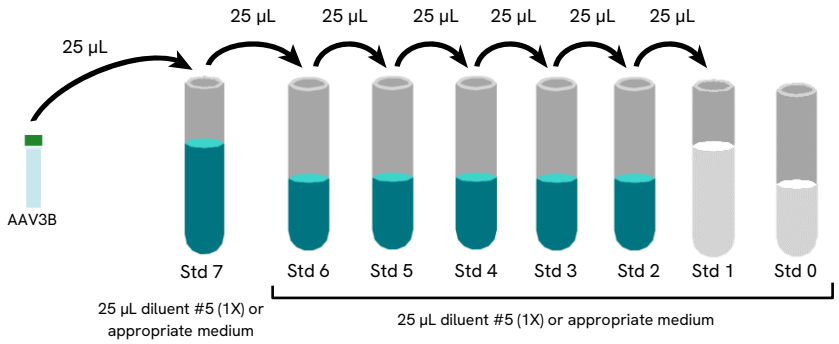
A recommended standard dilution procedure is listed and illustrated below

Dilute the standard stock solution 2-fold with diluent #5 (1X) to prepare high standard (Std 7): e.g. take 25 µL of standard stock solution and add it to 25 µL of diluent #5 (1X). Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/2 serial dilutions as follows:

- Dispense 25 µL of diluent #5 (1X) in each vial from Std 6 to Std 0.
- Add 25 µL of standard to 25 µL of diluent #5 (1X), mix gently and repeat the 1/2 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	WORKING SOLUTIONS
Standard Stock solution	Thawed stock solution	1.20E+12 VP/mL
Standard 7	25 µL stock solution + 25 µL Diluent #5 (1X)	6.00E+11 VP/mL
Standard 6	25 µL standard 7 + 25 µL Diluent #5 (1X)	3.00E+11 VP/mL
Standard 5	25 µL standard 6 + 25 µL Diluent #5 (1X)	1.50E+11 VP/mL
Standard 4	25 µL standard 5 + 25 µL Diluent #5 (1X)	7.50E+10 VP/mL
Standard 3	25 µL standard 4 + 25 µL Diluent #5 (1X)	3.75E+10 VP/mL
Standard 2	25 µL standard 3 + 25 µL Diluent #5 (1X)	1.88E+10 VP/mL
Standard 1	25 µL standard 2 + 25 µL Diluent #5 (1X)	9.38E+09 VP/mL
Standard 0	25 µL Diluent #5 (1X)	0

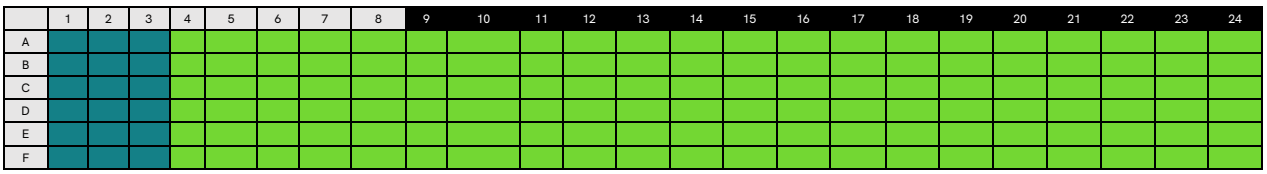
To prepare samples

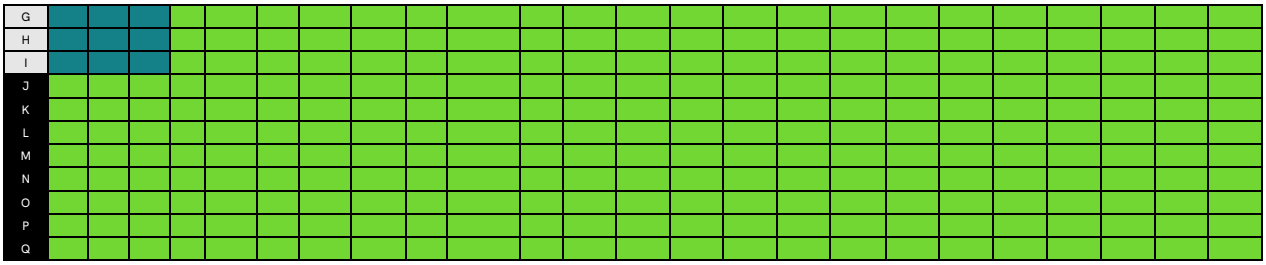
- Each well requires 5 µL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 7) must be diluted diluent #5 (1X) or in your appropriate sample medium.
- In order to measure AAV3B in cell lysates, cells must be lysed with Lysis Buffer #3 (1X) for 30 min at RT under gentle shaking.

ASSAY PROTOCOL

		STANDARD (STD 0 – STD 7)	SAMPLES
Step 1		Dispense 5 µL of each AAV3B standard (Std 0 - Std 7) into each standard well. And add 5 µL of diluent #5 (1X)	Dispense 5 µL of each sample into each sample well. And add 5 µL of diluent #5 (1X)
Step 2		Add 5 µL of Premix 1 with acceptor working solution to all wells	
Step 3		Add 5 µL of Premix 2 with donor working solution to all wells.	
Step 4		Seal the plate and incubate Overnight at RT Following incubation, the signal remains stable over a period of 48 hours.	
Step 5		Remove the plate sealer and read on an HTRF® compatible reader	

	1	2	3	4	5	6
A	5 µL Std 0 (Negative control) 5 µL of diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well A1**	**Repeat Well A1**	5 µL sample 1 5 µL diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well A4**	**Repeat Well A4**
B	5 µL Std 1 5 µL of diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well B1**	**Repeat Well B1**	5 µL sample 2 5 µL diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well B4**	**Repeat Well B4**
C	5 µL Std 2 5 µL of diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well C1**	**Repeat Well C1**	5 µL sample 3 5 µL diluent #5 5 µL Premix 1 5 µL Premix 2	**Repeat Well C4**	**Repeat Well C4**
D	5 µL Std 3 5 µL of diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well D1**	**Repeat Well D1**	5 µL sample 4 5 µL diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well D4**	**Repeat Well D4**
E	5 µL Std 4 5 µL of diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well E1**	**Repeat Well E1**	5 µL sample 5 5 µL diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well E4**	**Repeat Well E4**
F	5 µL Std 5 5 µL of diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well F1**	**Repeat Well F1**	5 µL sample 6 5 µL diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well F4**	**Repeat Well F4**
G	5 µL Std 6 5 µL of diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well G1**	**Repeat Well G1**	5 µL sample ... 5 µL diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well G4**	**Repeat Well G4**
H	5 µL Std 7 5 µL of diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well H1**	**Repeat Well H1**	5 µL sample ... 5 µL diluent #5 5 µL Premix 1 **5 µL Premix 2**	**Repeat Well H4**	**Repeat Well H4**





DATA REDUCTION & INTERPRETATION

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 delta ratio of the acceptor and donor emission signals for each individual well. The Standard 0 (Negative control) plays the role of an internal assay control.

delta Ratio = Ratio Standard or sample - Ratio Standard 0

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

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

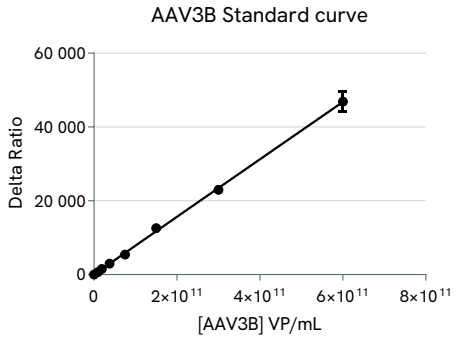
For more information about data reduction, please visit our website.

RESULTS

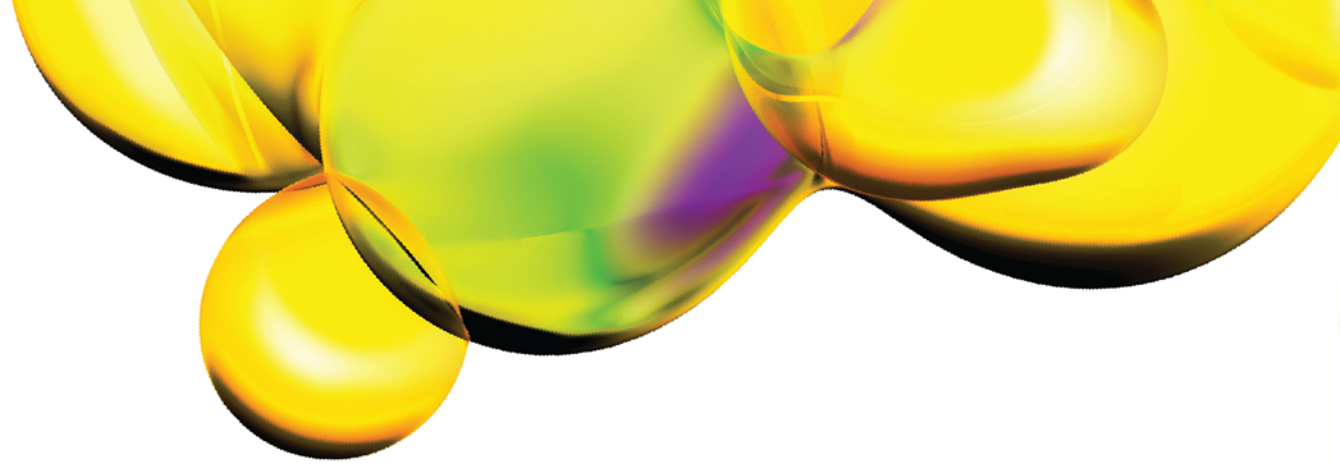
This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.
Results may vary from one HTRF® compatible reader to another.
Standard curve fitting with the 4 Parameter Logistic (4PL with $1/Y^2$) model.

(For more information about curve fitting please visit our website)

		Ratio (1)	Ratio (2)	CV% (3)
Standard 0	Negative control	925	0	0%
Standard 1	9.38E+09 VP/mL	1583	657	9.3%
Standard 2	1.88E+10 VP/mL	2471	1545	3.7%
Standard 3	3.75E+10 VP/mL	3920	2995	4.4%
Standard 4	7.50E+10 VP/mL	6349	5423	7.4%
Standard 5	1.50E+11 VP/mL	13491	12565	8.8%
Standard 6	3.00E+11 VP/mL	23883	22957	3.6%
Standard 7	6.00E+11 VP/mL	47812	46886	5.9%



REACH European regulations and compliance.
REACH European regulations and compliance This product and/or some of its components include a Triton concentration of 0.1% or more and as such, it is concerned by the REACH European regulations. We recommend researchers using this product to act in compliance with REACH and in particular: to only use the product for in vitro research in appropriate and controlled premises by qualified researchers, ii) to ensure the collection and the treatment of subsequent waste, and iii) to make sure that the total amount of Triton handled does not exceed 1 ton per year
This product contains material of biologic origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage. The use of the cell line will be done with appropriate safety and handling precautions to minimize health and environmental impact. Remaining disclaimer.



Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

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