MANUAL

Technology: HTRF® Biomarkers

HTRF AAV9 Capsid detection Kit

Part number	64AAV9PEG	64AAV9PEH
Test size	500 tests	10,000 tests

Storage: ≤-60°C or below

Version: 02 **Date:** March 2024

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Adeno-associated virus serotype 9 (AAV9) 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. AAV9 is an efficient vector for lung, liver, 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, AAV9 capsid is detected in a sandwich assay by using anti-AAV9 antibody labelled with Europium cryptate (donor), and anti-AAV9 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 AAV9 capsid concentration.

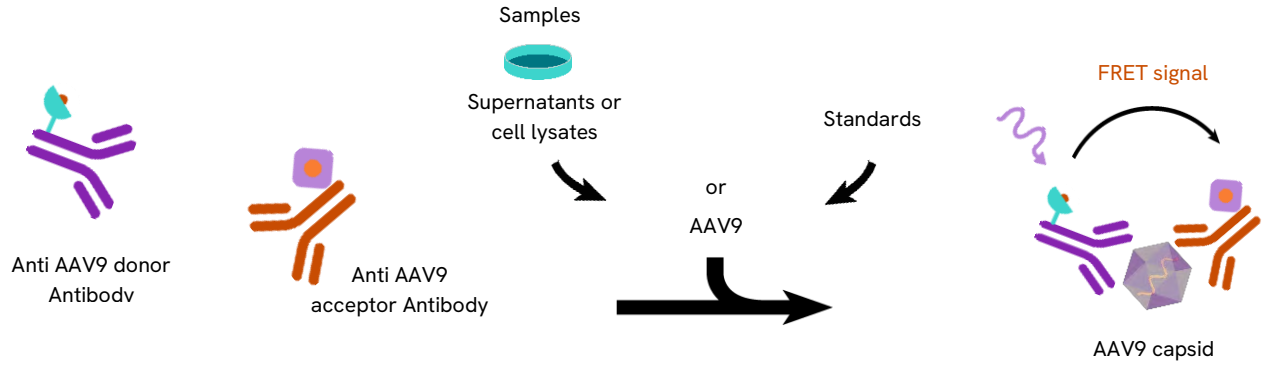
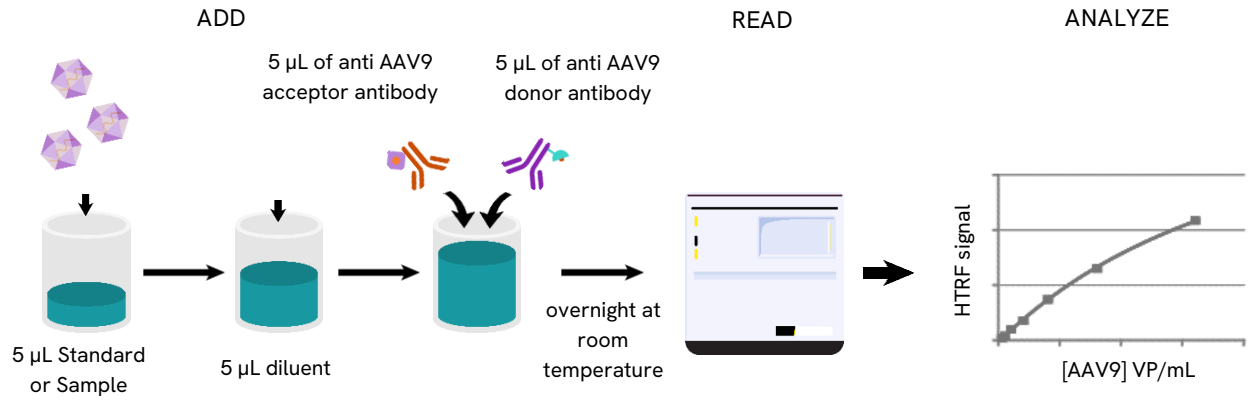


Figure 1: Principle of HTRF AAV9 sandwich assay.

PROTOCOL AT A GLANCE



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

MATERIAL PROVIDED

KIT COMPONENTS	500 TESTS*			10,000 TESTS*		
AAV9 Standard Frozen		green cap	1 vial 4.00E+11 VP/mL		green cap	2 vials 4.00E+11 VP/mL
AAV9 Eu cryptate Antibody 20X		orange cap	1 vial 125 µL		red cap	2 vials 1.25 mL
AAV9 d2 Antibody 20X		blue cap	1 vial 125 µL		purple cap	2 vials 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 for Standard and ≤-16°C for antibody solutions.
- 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

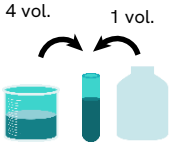
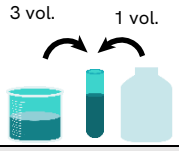
REAGENT PREPARATION

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.
- AAV5 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.

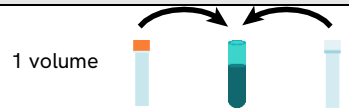
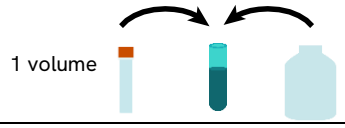
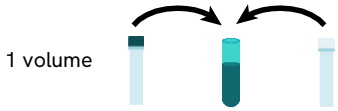
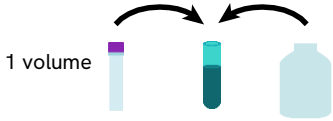
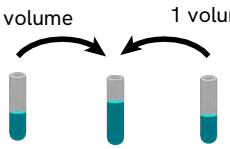
To prepare reagent stock solutions

500 TESTS		10.000 TESTS
Anti AAV9 Eu Cryptate antibody		
Thaw the AAV9 Eu Cryptate antibody. Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.		Thaw the AAV9 Eu Cryptate antibody. Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
Anti AAV9 d2 antibody		
Thaw the AAV9 d2 antibody. Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.		Thaw the AAV9 d2 antibody. Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
AAV9 capsid Standard		
Thaw the AAV9 standard to obtain 4.00E+11 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 AAV9 standard to obtain 4.00E+11 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 Anti AAV9 d2 antibody and 5 µL of of Anti AAV9 Eu Cryptate antibody.
Prepare the antibody solutions in separate vials.

<table>
<tr><th>500 TESTS</th><th>10,000 TESTS</th></tr>
<tr><td colspan="2">AAV9 Eu Cryptate antibody</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>Dilute 20-fold the 20X stock solution (thawed reagent) of AAV9 Eu Cryptate antibody with Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 19 volumes of detection buffer (e.g. 125 µL of Eu Cryptate antibody stock solution + 2375 µL of detection buffer).</td><td>Dilute 20-fold the 20X stock solution (thawed reagent of AAV9 Eu Cryptate antibody with Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 19 volumes of detection buffer (e.g. 2.5 mL of Eu Cryptate antibody stock solution + 47.5 mL of detection buffer).</td></tr>
<tr><td colspan="2">AAV9 d2 antibody</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>Dilute 20-fold the 20X stock solution (thawed reagent) of AAV9 d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 19 volumes of detection buffer (e.g. 125 µL of d2 antibody stock solution + 2375 µL of detection buffer).</td><td>Dilute 20-fold the 20X stock solution (thawed reagent) of AAV9 d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 19 volumes of detection buffer (e.g. 2.5 mL of d2 antibody stock solution + 47.5 mL of detection buffer).</td></tr>
<tr><td colspan="2">Antibody mix</td></tr>
<tr><td>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. 2.5 mL of d2 antibody + 2.5 mL of Cryptate antibody).</td><td> 
</td><td>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. 50 mL of d2 antibody + 50 mL of Cryptate antibody).</td></tr>
</table>

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.

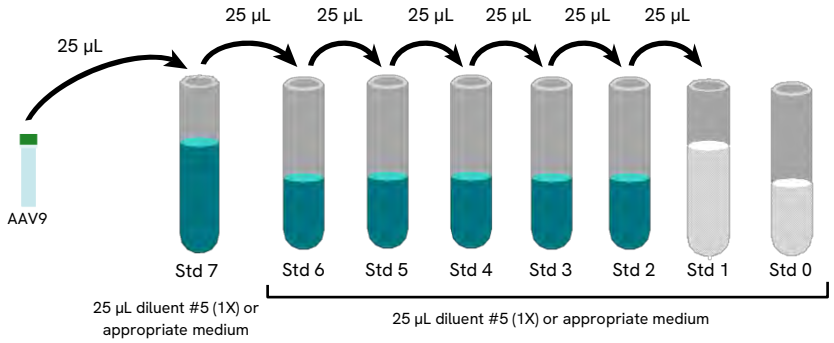
A recommended standard dilution procedure is listed and illustrated below

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	4.00E+11 VP/mL
Standard 7	25 µL stock solution + 25 µL Diluent #5 (1X)	2.00E+11 VP/mL
Standard 6	25 µL standard 7 + 25 µL Diluent #5 (1X)	1.00E+11 VP/mL
Standard 5	25 µL standard 6 + 25 µL Diluent #5 (1X)	5.00E+10 VP/mL
Standard 4	25 µL standard 5 + 25 µL Diluent #5 (1X)	2.50E+10 VP/mL
Standard 3	25 µL standard 4 + 25 µL Diluent #5 (1X)	1.25E+10 VP/mL
Standard 2	25 µL standard 3 + 25 µL Diluent #5 (1X)	6.25E+09 VP/mL
Standard 1	25 µL standard 2 + 25 µL Diluent #5 (1X)	3.13E+09 VP/mL
Standard 0	25 µL Diluent #5 (1X)	0

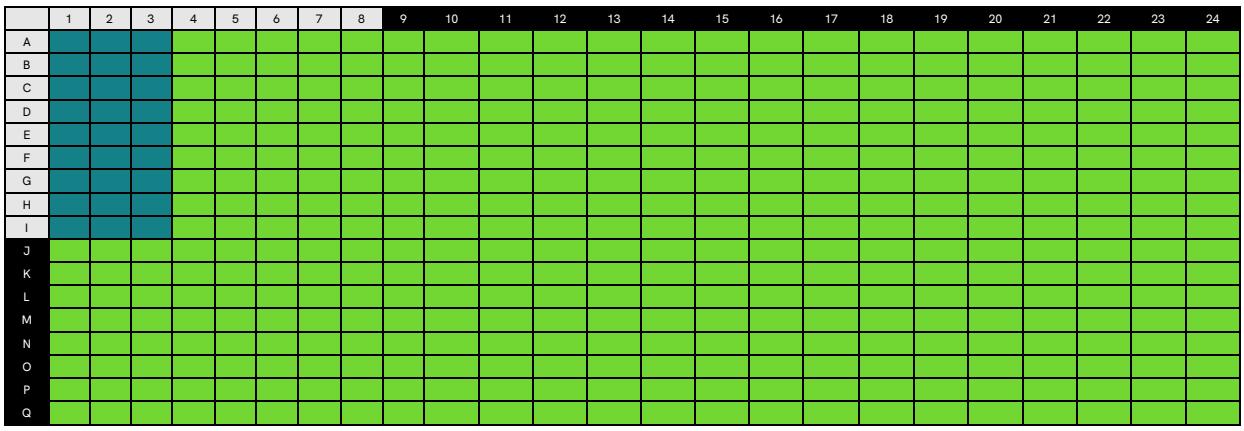
To prepare samples

- 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 AAV9 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 AAV9 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 AAV9 d2 antibody working solution to all wells	
Step 3		Add 5 µL of AAV9 Eu Cryptate antibody 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 AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well A1	Repeat Well A1	5 µL sample 1 5 µL diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well A4	Repeat Well A4
B	5 µL Std 1 5 µL of diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well B1	Repeat Well B1	5 µL sample 2 5 µL diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well B4	Repeat Well B4
C	5 µL Std 2 5 µL of diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well C1	Repeat Well C1	5 µL sample 3 5 µL diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well C4	Repeat Well C4
D	5 µL Std 3 5 µL of diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well D1	Repeat Well D1	5 µL sample 4 5 µL diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well D4	Repeat Well D4
E	5 µL Std 4 5 µL of diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well E1	Repeat Well E1	5 µL sample 5 5 µL diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well E4	Repeat Well E4
F	5 µL Std 5 5 µL of diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well F1	Repeat Well F1	5 µL sample 6 5 µL diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well F4	Repeat Well F4
G	5 µL Std 6 5 µL of diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well G1	Repeat Well G1	5 µL sample ... 5 µL diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well G4	Repeat Well G4
H	5 µL Std 7 5 µL of diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	Repeat Well H1	Repeat Well H1	5 µL sample ... 5 µL diluent #5 5 µL AAV9 d2 5 µL AAV9 Eu Cryptate	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.

$$\text{delta Ratio} = \text{Ratio Standard or sample} - \text{Ratio Standard 0}$$

3) 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 our website.

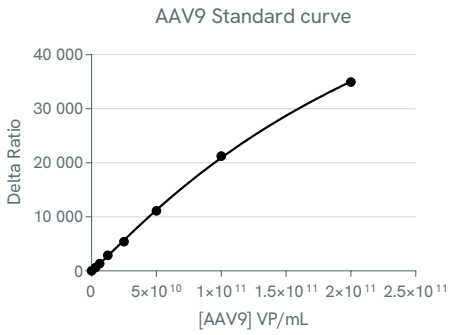
RESULTS

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

		Ratio (1)	Delta Ratio (2)	CV% (3)
Std 0	Negative control	961	0	0%
Std 1	3.13E+09 VP/mL	1567	606	7%
Std 2	6.25E+09 VP/mL	2302	1341	3%
Std 3	1.25E+10 VP/mL	3844	2883	4%
Std 4	2.50E+10 VP/mL	6384	5423	1%
Std 5	5.00E+10 VP/mL	12070	11109	3%
Std 6	1.00E+11 VP/mL	22203	21242	3%
Std 7	2.00E+11 VP/mL	35900	34939	1%



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.

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