MANUAL

Technology: HTRF® Biomarkers

HTRF Human Serum Albumin Kit

Part number:	6FHSAPEG	6FHSAPEH
Test size	500 tests	10,000 tests

Storage: ≤ -20°C

Version: 05 Date: January 2024

ASSAY PRINCIPLE

This assay is intended for the quantitative determination of HSA using the HTRF® technology. HSA can be measured directly from cell supernatants or purified solutions.

As shown in the diagram below, HSA is detected in a sandwich assay format using 2 different specific monoclonal antibodies, one labelled with Eu^{3+}-Cryptate (donor) and the second 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 (665nm).

The two conjugates bind to the antigen present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen–antibody complexes formed and therefore to the HSA concentration.

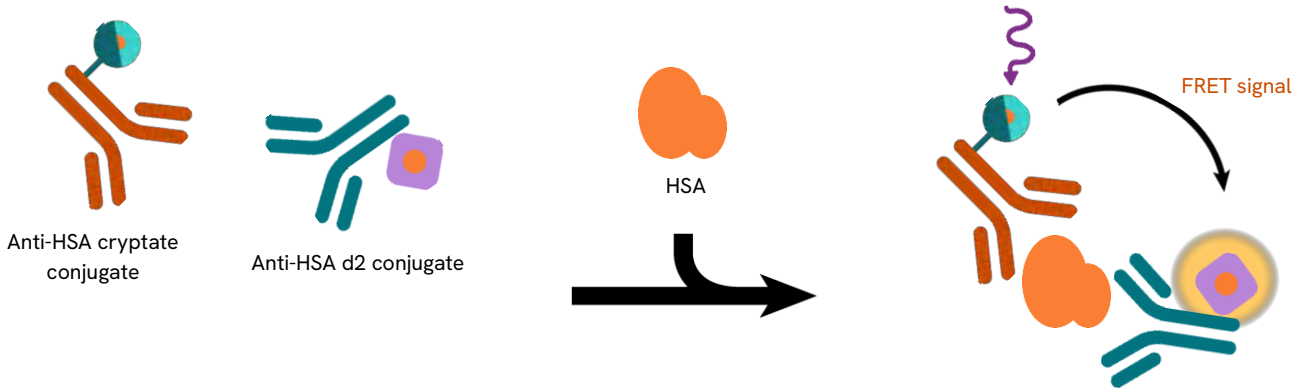
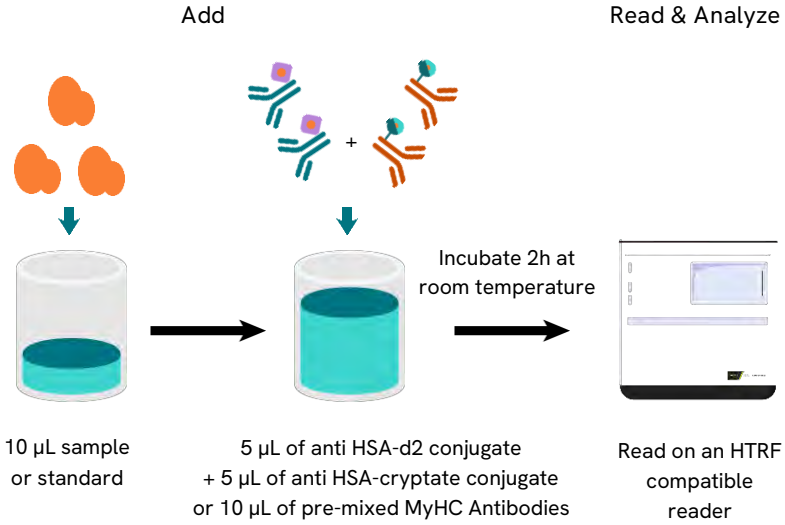


Figure 1: Principle of HTRF HSA sandwich assay

PROTOCOL AT A GLANCE



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

MATERIAL PROVIDED

KIT COMPONENTS	STORAGE	500 TESTS			10,000 TESTS		
HSA Standard	≤-20°C		green cap	10 µL/vial 100 µg/mL		green cap	10 µL/vial 100 µg/mL
Anti-HSA Ab-d2 conjugate	≤-20°C		blue cap	25 µL/vial		purple cap	500 µL/vial
Anti-HSA Ab-Eu^{3+}-Cryptate conjugate	≤-20°C		orange cap	25 µL/vial		red cap	500 µL/vial
Diluent	4°C to -20°C*		white cap	20 mL/vial		white cap	20 mL/vial
Conjugate buffer	4°C to -20°C*		red cap	13 mL/vial		red cap	2 vials 50 mL/vial

* *The diluent and conjugate buffer shipped frozen, but can be stored at 2-8°C in your premises.

REAGENT PREPARATION

HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 and Cryptate conjugates will impair the assay quality.

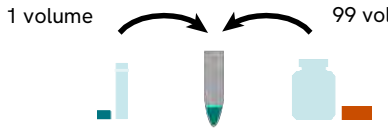
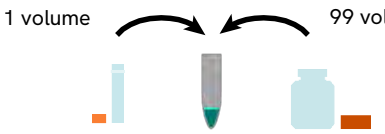
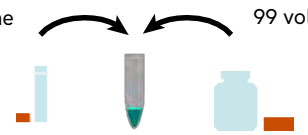
For an accurate quantitative determination of sample, dilution must be carried out with the medium used for preparing the samples (i.e. diluent, culture medium or any other compatible medium).

Standard and conjugates may be frozen and thawed once: to avoid freeze/thaw cycles it is recommended to dispense remaining stock solutions of standard and conjugates into disposable plastic vials for storage at –20°C or below.

- Thaw all reagents at room temperature, allow them to warm up
- Prepare the working solutions from stock solutions by following the instructions below.

Preparation of conjugate working solutions

Determine the amount of conjugate needed for the experiment. Each well requires 4 µL of each conjugate.

500 TESTS KIT	10,000 TESTS KIT
Anti-HSA-d2 conjugate	
	
Prepare a 100X diluted solution using the conjugate buffer: e.g. take 25 µL of conjugate stock solution and add it to 2475 µL of conjugate buffer.	Prepare a 100X diluted solution using the conjugate buffer: e.g. take 500 µL of conjugate stock solution and add it to 49.5 mL of conjugate buffer.
Anti-HSA-Eu^{3+}-Cryptate conjugate	
	
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Standard curve preparation

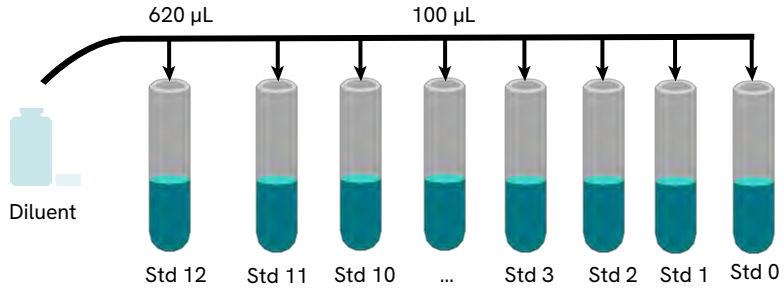
Determine how many samples and replicates to be tested. Each well requires 10 µL of sample or standard.
NB: If the sample to test is a cell supernatant, replace the diluent by culture medium.

STANDARD	PREPARATION	WORKING CONCENTRATION (ng/mL)
Standard 12	5 µL Stock solution + 620 µL diluent	800
Standard 11	100µl Std 12 + 100µl diluent	400
Standard 10	100µl Std 11 + 100µl diluent	200
Standard 9	100µl Std 10 + 100µl diluent	100
Standard 8	100µl Std 9 + 100µl diluent	50
Standard 7	100µl Std 8 + 100µl diluent	25
Standard 6	100µl Std 7 + 100µl diluent	12.50
Standard 5	100µl Std 6 + 100µl diluent	6.25
Standard 4	100µl Std 5 + 100µl diluent	3.13
Standard 3	100µl Std 4 + 100µl diluent	1.56
Standard 2	100µl Std 3 + 100µl diluent	0.78
Standard 1	100µl Std 2 + 100µl diluent	0.39
Standard 0	100µl diluent	0

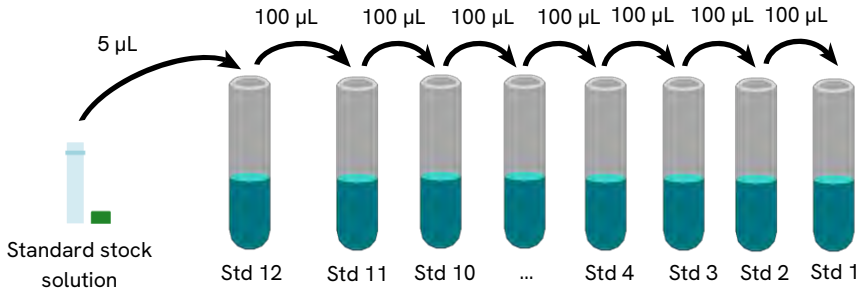
A recommended standard dilution procedure is listed and illustrated below.

- Dilute the standard stock solution 125-fold with diluent; this yields the high standard (Std 12: 800 ng/mL) for the top of the curve.
  - In practice: take 5 µL of this pre-dilution and add it to 620 µL of diluent. Mix gently.
- Use the high standard (Std 12) to prepare the standard curve using 1/2 serial dilutions as follows:
  - Dispense 100µL of diluent in each vial from Std 11 to Std 1.
  - Add 100µL of standard to 100µL of diluent, mix gently and repeat the 1/2 serial dilution to make standard solutions: 400, 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 ng/mL.
    This will create 12 standards for the analyte.
    Std 0 (negative control) is diluent alone.

Step 1: dispense diluent in each vial.

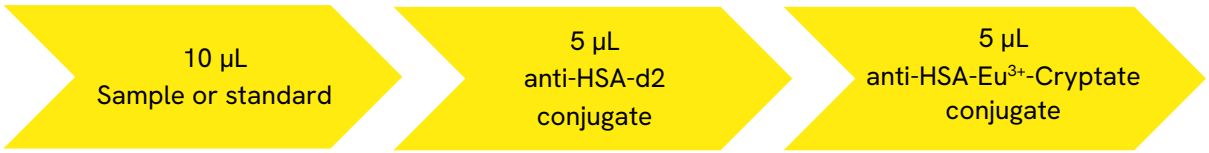


Step 2: dilute standards



ASSAY PROTOCOL

Dispense the reagents in the following order:



Please Note: It is possible to pre-mix the two conjugates just before dispensing and add 10 µl of this mix.

- Cover the plate with a plate sealer.
- Incubate for 2 hours at RT
- Remove the plate sealer and read the fluorescence emission at two different wavelengths (665nm and 620nm) on an HTRF® compatible reader.

For HTRF certified reader

For more information about HTRF® compatible readers and for set-up recommendations, please visit our website.

	Assays controls			
	Negative control	Cryptate control	Buffer control	Sample / Std
	used to calculate the delta F%	*used to check the Cryptate signal at 620 nm*	*used to check background fluorescence*	
Sample / Std	-	-	-	10 µL
Diluent	10 µL	10 µL	10 µL	-
Anti-HSA-d2 conjugate	5 µL	-	-	5 µL
Anti-HSA-Eu^{3+}-Cryptate conjugate	5 µL	5 µL	-	5 µL
Conjugate buffer	-	5 µL	10 µL	-

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

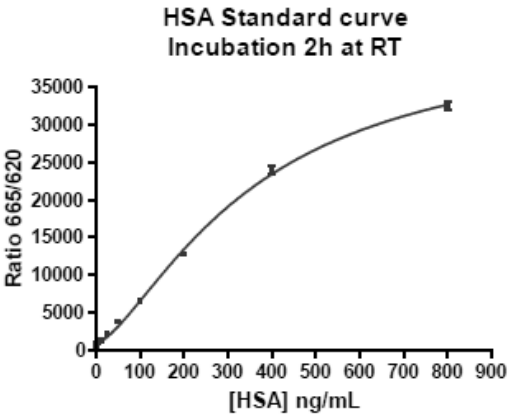
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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

RESULTS

These data must not be substituted for that obtained in the laboratory and should be considered only as an example. Results may vary from one HTRF® compatible reader to another.
The assay standard curve was generated with a four parameter logistic (4PL) curve-fit and is drawn up by plotting the Ratio 665/620 versus the analyte concentration:

Standard ng/ml		Ratio (1)	CV % (2)
Standard 0	Negative control	476	3.7%
Standard 1	0.390625	558	3.7%
Standard 2	0.78125	612	2.9%
Standard 3	1.5625	787	2.5%
Standard 4	3.125	861	11.3%
Standard 5	6.25	946	5.4%
Standard 6	12.5	1249	1.6%
Standard 7	25	2086	5.4%
Standard 8	50	3667	4.7%
Standard 9	100	6491	1.5%
Standard 10	200	12 721	1.1%
Standard 11	400	23 973	2.4%
Standard 12	800	32 450	1.7%



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

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