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PerkinElmer For the Better | cisbio

Human Serum Albumin

For in vitro research use only
Reagent storage temperature: -20°C or below

www.cisbio.com

Packaging details :

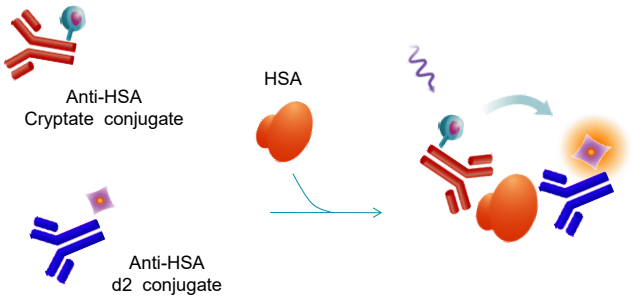
	384-well low volume plate (20 µl)
6FHSAPEH	10 000 tests

Product information

Document reference: 6FHSAPEH-Rev04-Dec.2020

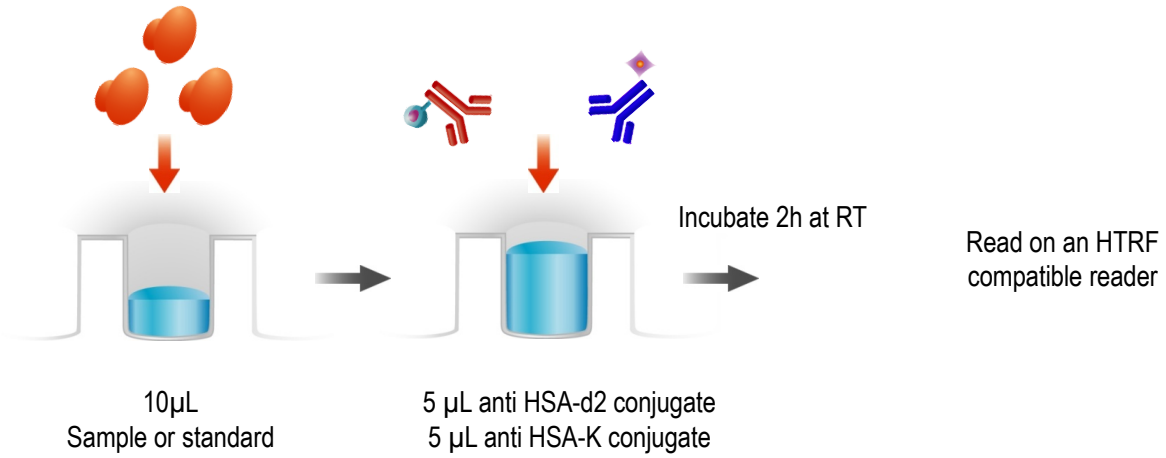
1. Assay description

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 to the right, 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).

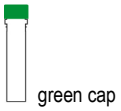
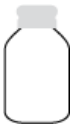
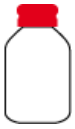


The two conjugates bind to the antigen present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen–antibody complexes formed and therefore to the HSA concentration.

2. Protocol at a glance



3. HTRF® reagents

	HSA Standard	Anti-HSA Ab-d2 conjugate	Anti-HSA Ab-Eu^{3+}-Cryptate conjugate	Diluent	Conjugate buffer
					
Stock solution	10 µL/vial 100 µg/mL	500 µL/vial	500 µL/vial	20 mL/vial	2 x 50 mL/vial
Storage	-20°C or below	-20°C or below	-20°C or below	4°C to -20°C*	4°C to -20°C*

* Diluent and Conjugate buffer are shipped frozen, but can be stored at 2-8°C in your premises.

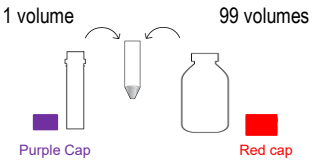
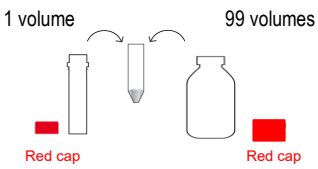
4. Reagent preparation

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.
- Prepare the working solutions from stock solutions (§3) by following the instructions below.

4.1. Preparation of conjugate working solutions

Determine the amount of conjugate needed for the experiment. Each well requires 5 µL of each conjugate.

Anti-HSA-d2 conjugate	Anti-HSA-Eu^{3+}-Cryptate conjugate
	
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.	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.

4.2. Standard curve preparation

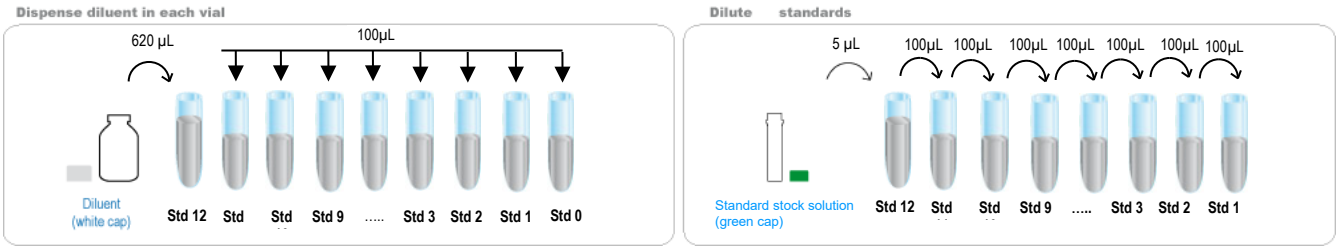
Determine how many samples and replicates to be tested. Each well requires 10 µL of sample or standard.
NB: If the sample to test is a cell supernatant, replace the diluent by culture medium.

Standards	Working concentration (ng/mL)	Preparation
Std 12	800	5 µL Stock solution + 620 µL diluent
Std 11	400	100 µL Std 12 + 100 µL diluent
Std 10	200	100 µL Std 11 + 100 µL diluent
Std 9	100	100 µL Std 10 + 100 µL diluent
Std 8	50	100 µL Std 9 + 100 µL diluent
Std 7	25	100 µL Std 8 + 100 µL diluent
Std 6	12.50	100 µL Std 7 + 100 µL diluent
Std 5	6.25	100 µL Std 6 + 100 µL diluent
Std 4	3.13	100 µL Std 5 + 100 µL diluent
Std 3	1.56	100 µL Std 4 + 100 µL diluent
Std 2	0.78	100 µL Std 3 + 100 µL diluent
Std 1	0.39	100 µL Std 2 + 100 µL diluent
Std 0	0	100 µL diluent

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 100 µL of diluent in each vial from Std 11 to Std 1.*
- *Add 100 µL of standard to 100 µL 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.



5. Assay protocol

Dispense the reagents in the following order:

10 µL Sample or standard → 5 µL Anti-HSA-d2 conjugate → 5 µL Anti-HSA-Eu^{3+}-Cryptate conjugate

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 at 18-22°C for 2 hours.
→ Remove the plate sealer and,
→ Read the fluorescence emission at two different wavelengths (665nm and 620nm) on an HTRF® compatible reader.

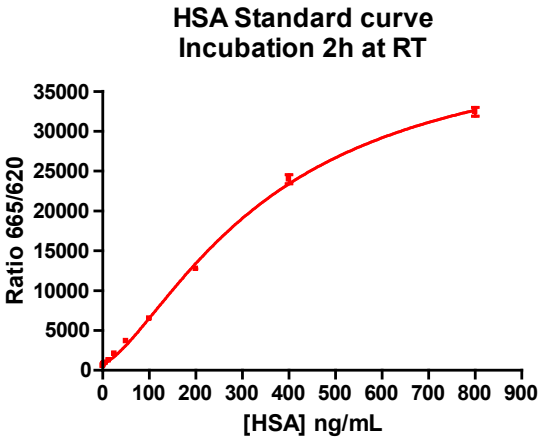
For more information about HTRF® compatible readers, please visit our website at: www.cisbio.com/compatible-readers

		Assay 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	-

6. Data reduction

These data must not be substituted for that obtained in the laboratory and should be considered only as an example (readouts on PHERAstar^{FS}). 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:

Standards ng / mL	Ratio (1)	CV % (2)
Std 0 - Negative control	476	3.7%
Std 1 – 0.390625	558	3.7%
Std 2 – 0.78125	612	2.9%
Std 3 – 1.5625	787	2.5%
Std 4 – 3.125	861	11.3%
Std 5 – 6.25	946	5.4%
Std 6 – 12.5	1249	1.6%
Std 7 – 25	2086	5.4%
Std 8 – 50	3667	4.7%
Std 9 – 100	6491	1.5%
Std 10 – 200	12721	1.1%
Std 11 – 400	23973	2.4%
Std 12 – 800	32450	1.7%



Ratio (1)	$\frac{\text{Signal}_{665nm}}{\text{Signal}_{620nm}} \times 10^4$	Ratio must be calculated for each individual well.
CV% (2)	$\frac{\text{Standard deviation}}{\text{Mean ratio}} \times 100$	The mean and standard deviation can then be worked out from ratio replicates.
For more information about data reduction, please visit our website at: www.cisbio.com/htrf-ratio-and-data-reduction		

To obtain additional information or support, please contact your technical support team (htrfservices@cisbio.com).