MANUAL

Technology: HTRF® Biomarkers

HTRF Human Apolipoprotein A1 kit

Part number:	**64APAPEG**	**64APAPEH**
Test size	500 tests	10,000 tests

Storage: ≤ 60°C

Version: 05 **Date:** January 2024

For research only. Not for use in diagnostic procedures

ASSAY PRINCIPLE

This assay is intended for the quantitative determination of Human Apolipoprotein A1 (Apo-A1) using HTRF® technology.

As shown in the diagram to the right, Human Apolipoprotein A1 is detected in a sandwich assay format using two different specific antibodies, one labeled with Eu^{3+}-Cryptate (donor) and the second with d2 (acceptor).

When these 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).

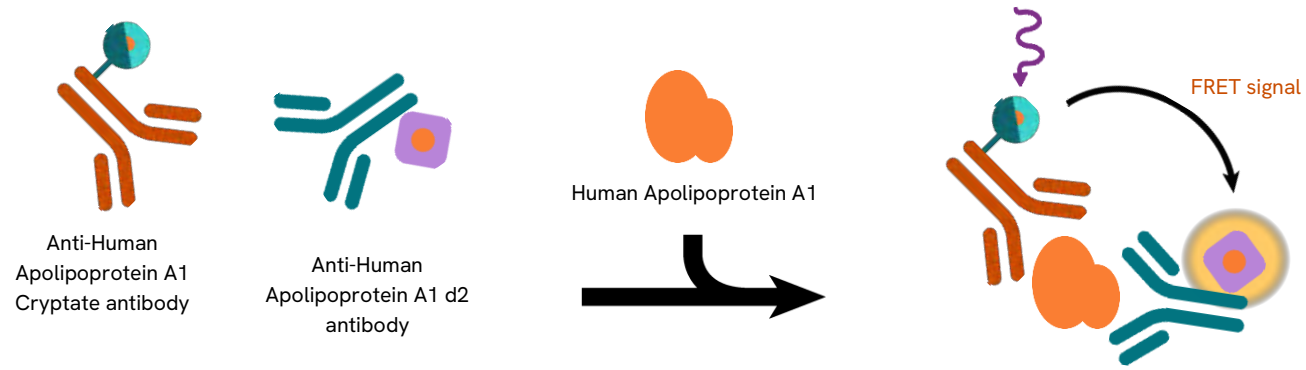
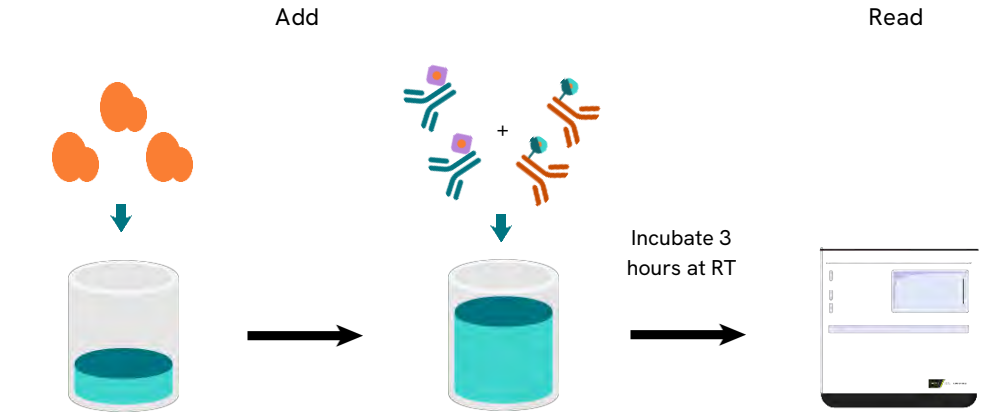


Figure 1: Principle of HTRF sandwich assay

The two antibodies 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 Human Apolipoprotein A1 concentration.

PROTOCOL AT A GLANCE



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

MATERIAL PROVIDED

KIT COMPONENTS		Human Apo-A1 Standard	Anti-Human Apo-A1 d2 antibody	Anti-Human Apo-A1 Eu^{3+}-Cryptate-antibody	Diluent	Detection buffer #3
500 TESTS	Tubes	Green cap	Blue cap	Red cap	White cap	Transparent cap
	Stock solution	200 µL/vial 10 µg/mL	50 µL/vial	50 µL/vial	20 mL/vial	7 mL/vial 2 vials
10,000 TESTS	Tubes	Green cap	Purple cap	Red cap	White cap	Red cap
	Stock solution	200 µL/vial 10 µg/mL	1 mL/vial	1 mL/vial	20 mL/vial	105 mL/vial 2 vials
STORAGE		≤ 60°C	≤ 60°C	≤ 60°C	2-8°C to -20°C*	2-8°C to -20°C*
CAUTION			Filter the Cryptate-antibody stock solution before use		Apolipoproteins are sticky components, we recommend to dilute them in the diluent provided in the kit, or in your own diluent containing over 0.5% BSA, or in cell culture medium supplemented with at least 2% FCS	Before use, we recommend to supplement the detection buffer with 0.02% triton X100.
			Antibody stock solutions must be frozen in liquid nitrogen			
		Use black plates only				

* Diluent and Detection buffer are shipped frozen, but can be stored at 2-8°C.

The Apo-A1 standard is prepared from human plasma. This was found to be negative for HBsAg, HIV-1, HIV-2, HIV-3, and HCV. As no test guarantees a product to be non-infectious, it is recommended to handle Apo-A1 solutions with the same precautions as potentially infectious specimens.

REAGENT PREPARATION

HTRF® reagent concentrations have been set for optimal assay performance. Any dilution or improper use of the d2 and Cryptate-antibodies will impair the quality of the assay.

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).

Antibodies may be frozen and thawed once: to avoid freeze/thaw cycles it is recommended that you dispense remaining stock solutions of antibodies into disposable plastic vials. Antibody stock solutions must be frozen in liquid nitrogen and stored at -60°C or below.

Please note, working solution preparation may differ between the 500 and the 10,000 test size kits.

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

One-plate assay protocol: cell-based

Dispense the reagents in the following order:

* Optimization of cell seeding densities is recommended.
** Time course study is recommended to determine the optimal stimulation time.
***The two working antibody solutions must be prepared in individual vials and can be mixed prior to dispense: add 1 volume of anti-Human Apolipoprotein A1-d2 antibody to 1 volume of anti-Human Apolipoprotein A1-Eu3+-cryptate antibody, and dispense 10 µL of the pre-mix anti-Human Apolipoprotein A1 antibodies.

- Cover the plate with a plate sealer.
- Incubate at RT for 3 hours.
- Remove the plate sealer and,
- Read the fluorescence emission at two different wavelengths (665nm and 620nm) on a compatible HTRF® reader.

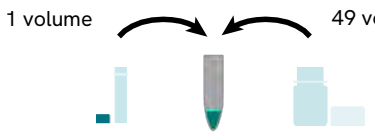
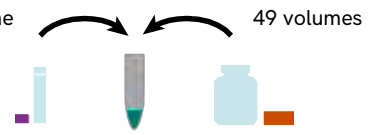
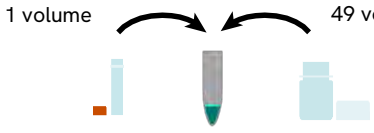
For HTRF certified reader

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

	Assays controls			
	Negative control	Cryptate control	Buffer control	Cells / Std
	used to calculate the delta F%	*used to check the Cryptate signal at 620 nm*	*used to check background fluorescence*	
Cells / Std	-	-	-	5 µL
Diluent	10 µL	10 µL	10 µL	5 µL
Anti-Human Apolipoprotein A1-d2 antibody	5 µL	-	-	5 µL
Anti-Human Apolipoprotein A1-Eu^{3+}-Cryptate antibody	5 µL	5 µL	-	5 µL
Detection buffer#3	-	5 µL	10 µL	-

Preparation of antibody working solutions

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

500 TESTS KIT	10,000 TESTS KIT
Anti-Human Apolipoprotein A1-d2 antibody	
	
Dilute 50-fold the stock solution of anti-human Apo-A1-d2 antibody with detection buffer: e.g. take 0.05 mL of d2-antibody stock solution and add it to 2.45 mL of detection buffer #3.	Dilute 50-fold the stock solution of anti-human Apo-A1-d2 antibody with detection buffer: e.g. take 1 mL of d2-antibody stock solution and add it to 49 mL of detection buffer #3.
Anti-Human Apolipoprotein A1-Eu^{3+}-Cryptate-antibody	
	
Dilute 50-fold the stock solution of anti-human Apo-A1-cryptate antibody with detection buffer e.g. take 0.05 mL of cryptate-antibody stock solution and add it to 2.45 mL of detection buffer #3.	Dilute 50-fold the stock solution of anti-human Apo-A1-cryptate antibody with detection buffer e.g. take 1 mL of cryptate-antibody stock solution and add it to 49 mL of detection buffer #3.

Standard curve preparation

Determine how many samples and replicates will be tested.

Each well requires 5 µL of sample or standard.

Please note: If the sample to test is a cell supernatant, replace the diluent by culture medium. As apolipoproteins are sticky components, we recommend to dilute them using buffers containing over 0.5%BSA or cell culture medium supplemented with at least 2% FCS.

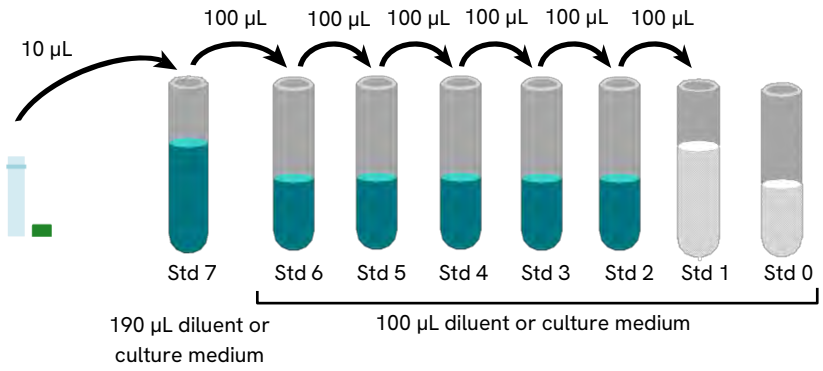
STANDARD	PREPARATION	WORKING CONCENTRATION (ng/mL)
Standard 7	200 µL standard stock solution	500
Standard 6	100 µL Std 7 + 100 µL diluent or culture medium	250
Standard 5	100 µL Std 6 + 100 µL diluent or culture medium	125
Standard 4	100 µL Std 5 + 100 µL diluent or culture medium	62.5
Standard 3	100 µL Std 4 + 100 µL diluent or culture medium	31.25
Standard 2	100 µL Std 3 + 100 µL diluent or culture medium	15.6
Standard 1	100 µL Std 2 + 100 µL diluent or culture medium	7.8
Standard 0	100 µL diluent or culture medium	0

A recommended standard dilution procedure is listed and illustrated below:

1) Pre-dilute the standard stock solution 20-fold with diluent. In practice: take 10µL of stock solution and add 190 µL of diluent or culture medium in the standard stock solution. Mix gently. This yields the high standard (Std 7: 500 ng/mL) for the top of the curve.
2) Prepare the following serial dilutions:

- Use the high standard (Std 7) to prepare the standard curve using 1/2 serial dilutions as follows:
  - Dispense 100µL of diluent in each vial from Std 6 to Std 1.
  - Add 100µL of standard to 100µL of diluent, mix gently and repeat the 1/2 serial dilution to make standard solutions: 250, 125, 62.5, 31.25, 15.6, 7.8 ng/mL.

This will create 7 standards for the analyte. Std 0 (Negative control) is diluent or culture medium alone.
The standard dilution procedure is listed and illustrated below.



Two-plate assay protocol: supernatant

Dispense the reagents in the following order:

5 µL cells* or standard → 5 µL compounds → Incubate at 37°C in CO2 atmosphere** → Transfer 2 µL of supernatant → 9 µL anti-Human Apolipoprotein A1-d2 antibody*** → 9 µL anti-Human Apolipoprotein A1-Eu^{3+}-cryptate

* Optimization of cell seeding densities is recommended.
** Time course study is recommended to determine the optimal stimulation time.
***The two working antibody solutions must be prepared in individual vials and can be mixed prior to dispense: add 1 volume of anti-Human Apolipoprotein A1-d2 antibody to 1 volume of anti-Human Apolipoprotein A1-Eu^{3+} cryptate antibody, and dispense 18 µL of the pre-mix anti-Human Apolipoprotein A1 antibodies.

- Cover the plate with a plate sealer.
- Incubate at RT for 3 hours.
- Remove the plate sealer and,
- Read the fluorescence emission at two different wavelengths (665nm and 620nm) on a compatible HTRF® reader.

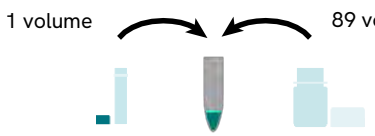
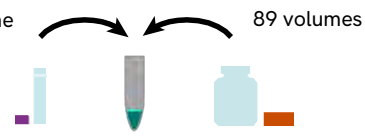
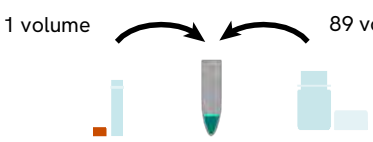
For HTRF certified reader

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

	Assays controls			
	Negative control	Cryptate control	Buffer control	Cells / Std
	used to calculate the delta F%	*used to check the Cryptate signal at 620 nm*	*used to check background fluorescence*	
Supernatant / Std	-	-	-	2 µL
Diluent	2 µL	2 µL	2 µL	-
Anti-Human Apolipoprotein A1-d2 antibody	9 µL	-	-	9 µL
Anti-Human Apolipoprotein A1-Eu^{3+}-Cryptate antibody	9 µL	9 µL	-	9 µL
Detection buffer#3	-	9 µL	18 µL	-

Preparation of antibody working solutions

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

500 TESTS KIT	10,000 TESTS KIT
Anti-Human Apolipoprotein A1-d2 antibody	
	
Dilute 90-fold the stock solution of anti-human Apo-A1-d2 antibody with detection buffer: e.g. take 0.01 mL of d2-antibody stock solution and add it to 0.89 mL of detection buffer #3.	Dilute 90-fold the stock solution of anti-human Apo-A1-d2 antibody with detection buffer: e.g. take 0.01 mL of d2-antibody stock solution and add it to 0.89 mL of detection buffer #3.
Anti-Human Apolipoprotein A1-Eu^{3+}-Cryptate-antibody	
	
Dilute 90-fold the stock solution of anti-human Apo-A1-cryptate antibody with detection buffer e.g. take 0.01 mL of cryptate-antibody stock solution and add it to 0.89 mL of detection buffer #3.	Dilute 90-fold the stock solution of anti-human Apo-A1-cryptate antibody with detection buffer e.g. take 0.01 mL of cryptate-antibody stock solution and add it to 0.89 mL of detection buffer #3.

Standard curve preparation

Determine how many samples and replicates will be tested.

Each well requires 5 µL of sample or standard.

Please note: If the sample to test is a cell supernatant, replace the diluent by culture medium. As apolipoproteins are sticky components, we recommend to dilute them using buffers containing over 0.5%BSA or cell culture medium supplemented with at least 2% FCS.

STANDARD	PREPARATION	WORKING CONCENTRATION (ng/mL)
Standard 7	200 µL standard stock solution	4 000
Standard 6	100 µL Std 7 + 100 µL diluent or culture medium	2 000
Standard 5	100 µL Std 6 + 100 µL diluent or culture medium	1 000
Standard 4	100 µL Std 5 + 100 µL diluent or culture medium	500
Standard 3	100 µL Std 4 + 100 µL diluent or culture medium	250
Standard 2	100 µL Std 3 + 100 µL diluent or culture medium	125
Standard 1	100 µL Std 2 + 100 µL diluent or culture medium	62.5
Standard 0	100 µL diluent or culture medium	0

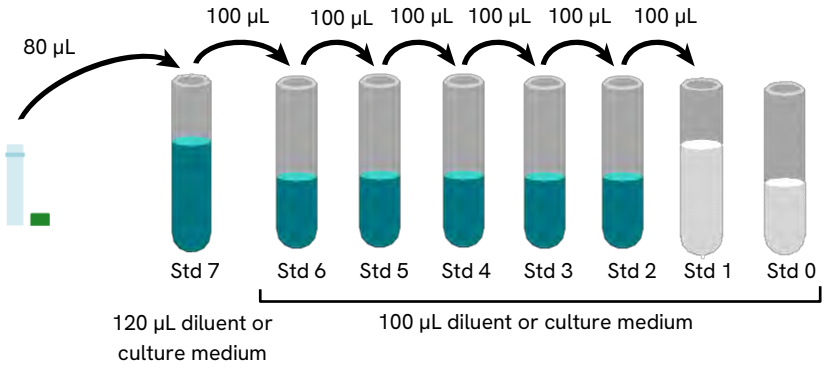
A recommended standard dilution procedure is listed and illustrated below:

1) Pre-dilute the standard stock solution 2.5-fold with diluent. In practice: take 40µL of stock solution and add 60 µL of diluent or culture medium in the standard stock solution. Mix gently. This yields the high standard (Std 7: 4,000 ng/mL) for the top of the curve.
2) Prepare the following serial dilutions:

- Use the high standard (Std 7) to prepare the standard curve using 1/2 serial dilutions as follows:
  - Dispense 100 µL of diluent in each vial from Std 6 to Std 1.
  - Add 100µL of standard to 100µL of diluent, mix gently and repeat the 1/2 serial dilution to make standard solutions: 2,000 - 1,000 – 500 - 250 - 125 - 62.5 ng/mL.

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

The standard dilution procedure is listed and illustrated below.



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.

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

3) Calculate the % delta F which reflects the signal to background of the assay. The negative control plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

$$\text{delta F (\%)} = \frac{\text{Ratio Standard or sample - Ratio Negative Control}}{\text{Ratio Negative Control}} \times 100$$

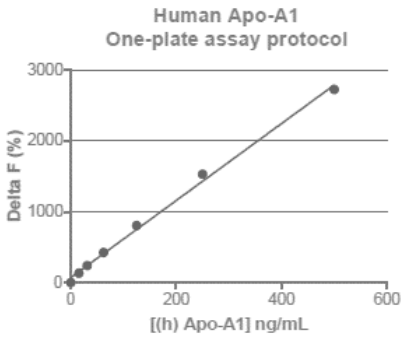
For more information about data reduction, please visit our website.

RESULTS

This 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.
Standard curve were prepared in DMEM (GIBCO, low glucose), 2% FBS medium (penicillin, streptomycin).
The assay standard curve is created by plotting delta F% versus the analyte concentration:

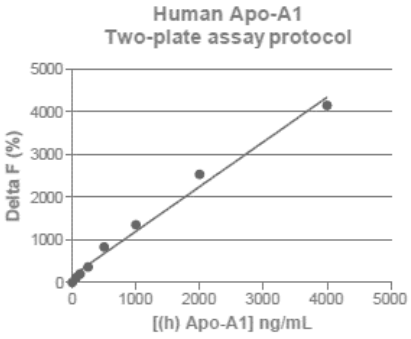
One-plate assay protocol

[Apo-A1 Std] (ng/mL)	Ratio (1)	CV % (2)	Delta F%
0	446	2%	0%
15.6	1 047	1%	135%
31.25	1 527	0%	242%
62.5	2 353	6%	427%
125	4 047	1%	806%
250	7 280	1%	1 531%
500	12 623	3%	2 727%



Two-plate assay protocol

[Apo-A1 Std] (ng/mL)	Ratio (1)	CV % (2)	Delta F%
0	479	3%	0%
62.5	997	3%	108%
125	1 422	4%	197%
250	2 218	3%	363%
500	4 468	7%	833%
1000	6 950	5%	1 351%
2 000	12 596	5%	2 530%
4 000	20 346	7%	4 148%



ANALYTICAL CHARACTERISTICS

Standardization

The HTRF® ApoA1 assay is calibrated against the ApoA1, A2 &B High Level Calibrator(Wako), based on IFCC Reference Preparation SP1-01.

Cross-reactivity

	Cross-reactivity %
Apo-A1	100%
Apo-B100	0.004%
Apo-A2	0.037%
Apo-C1	0.014%
Apo-C2	0.004%
Apo-C3	0.005%
Apo-E	0.004%

Detection limit

One-plate assay protocol: 0.3 ng/mL
Two-plate assay protocol (supernatant): 0.5 ng/mL

Extended range

One-plate assay protocol: 1,000 ng/mL
Two-plate assay protocol (supernatant): 8,000 ng/mL

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

www.revvity.com

revvity

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com