MANUAL

Technology: HTRF™ Biomarkers

HTRF Human Apolipoprotein B Detection Kit

Part number	64APOBPEG	64APOBPEH
Test size	500 tests	10,000 tests

Storage: ≤ 60°C

Version: 01 **Date:** April 2024

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Apolipoprotein B (ApoB) in supernatant and offers a fast alternative to ELISA.

The detection principle of this kit is based on HTRF™ technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, ApoB is detected in a sandwich assay by using anti-ApoB antibody labeled with Europium cryptate (donor), and anti ApoB antibody 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 an therefore to the ApoB concentration.

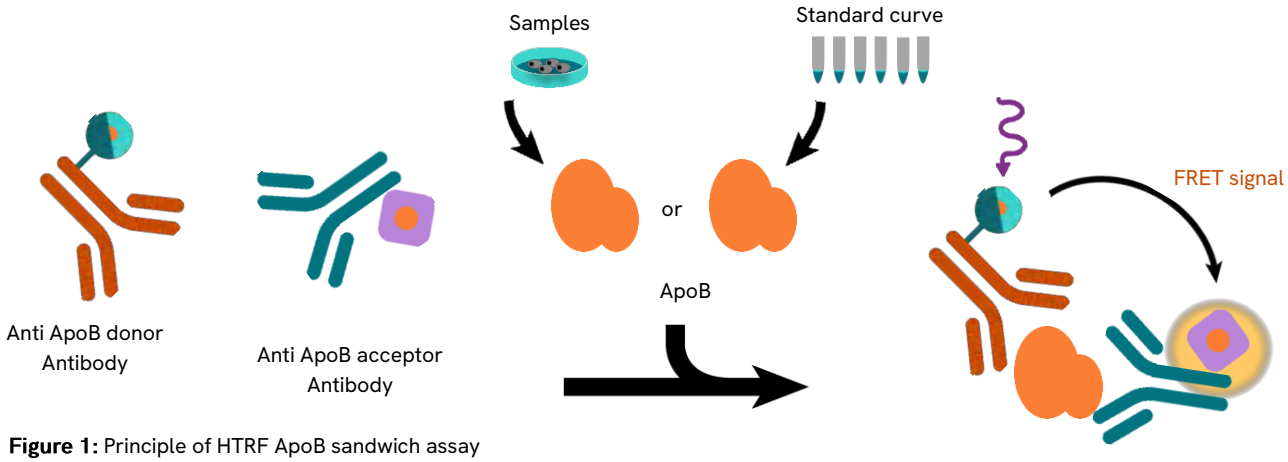
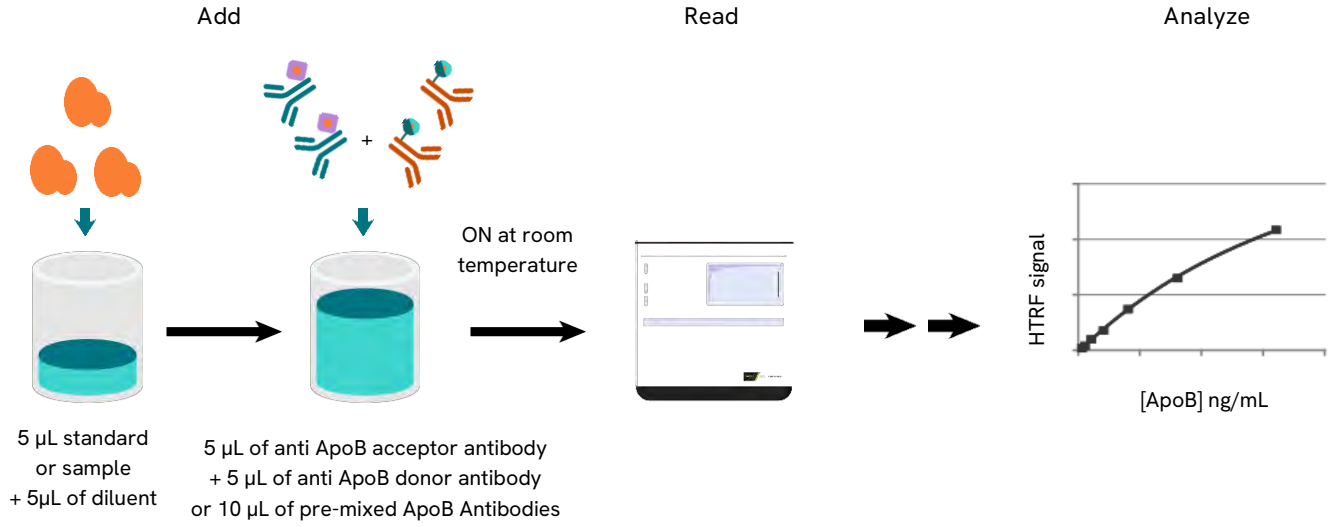


Figure 1: Principle of HTRF ApoB sandwich assay

PROTOCOL AT A GLANCE



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

MATERIAL PROVIDED

KIT COMPONENTS	500 TESTS*	10,000 TESTS*
ApoB Standard Frozen	1 vial – 150 µL 40 µg/mL	2 vials – 150 µL 40 µg/mL
ApoB Eu Cryptate Antibody Frozen 50X	1 vial 50 µL	1 vial 1 mL
ApoB d2 Antibody Frozen 50X	1 vial 50 µL	1 vial 1 mL
Diluent #1 Ready-to-use**	1 vial 20 mL	3 vials 20 mL
Detection Buffer* #3 Ready-to-use**	1 vial 7 mL	1 vial 100 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.

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. Use white plate only. 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

- If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
- Volume of Human ApoB standard aliquots should not be under 10 µL.

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.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- ApoB standards (for standard curve) must be prepared in diluent or in the same medium as the samples.

Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.

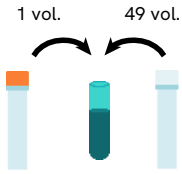
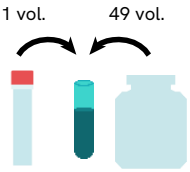
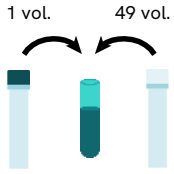
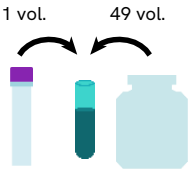
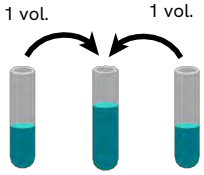
To prepare reagent stock solutions

500 TESTS			10,000 TESTS
Anti-ApoB Eu Cryptate antibody			
Thaw the ApoB Eu Cryptate antibody. Mix gently. This 50X 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 ApoB Eu Cryptate antibody. Mix gently. This 50X 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.
Anti-ApoB d2 antibody			
Thaw the ApoB d2 antibody. Mix gently. This 50X 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 ApoB d2 antibody . Mix gently. This 50X 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..
ApoB Standard			
Thaw the ApoB standard stock solution (40 µg/mL) at RT. Mix gently. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solution into disposable plastic vials for storage at -60°C or below.			Thaw the ApoB standard stock solution (40 µg/mL) at RT. Mix gently. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solution into disposable plastic vials for storage at -60°C or below
Diluent & Detection buffer			

The Diluent and the Detection buffer are ready-to-use.

To prepare antibody working solutions

Each well requires 5 µL of ApoB-Eu Cryptate Antibody and 5 µL of ApoB-d2 Antibody.
Prepare the two antibody solutions in separate vials.

500 TESTS		10,000 TESTS	
ApoB Eu Cryptate antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of ApoB Eu Cryptate antibody with Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of detection buffer (e.g. 20 µL of Eu Cryptate antibody stock solution + 980 µL of detection buffer).			Dilute 50-fold the 50X stock solution (thawed reagent) of ApoB Eu Cryptate antibody with Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of detection buffer (e.g. 0.4 mL of Eu Cryptate antibody stock solution + 19.6 mL of detection buffer).
ApoB d2 antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of ApoB d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of detection buffer (e.g. 20 µL of d2-antibody stock solution + 980 µL of detection buffer).			Dilute 50-fold the 50X stock solution (thawed reagent) of ApoB d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of detection buffer (e.g. 0.4 mL of d2 antibody stock solution + 19.6 mL of detection buffer).
Antibody Mix			
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. 1 mL of d2 antibody + 1 mL of Cryptate antibody).			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. 20 mL of d2 antibody + 20 mL of Cryptate antibody).

To prepare working standards solutions

- Each well requires 5 µL of standard.
- Dilute the standard stock solution serially with diluent #1
- If culture medium is used to dilute the standard, we recommend to supplement it with serum (2 to 10%) or BSA (0.2 to 1%) in order to avoid ApoB sticking to assay plates.
- 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 and in diluent #1.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

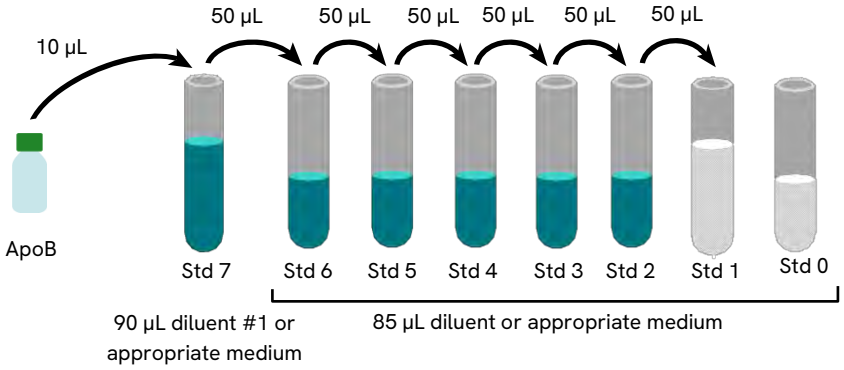
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 10-fold with diluent; this yields the Standard Max solution (4,000 ng/mL)

Use the high standard (Std 7) to prepare the standard curve using 1/2.7 serial dilutions as follows:

- Dispense 85 µL of diluent #1 in each vial from Std 6 to Std 0.
- Add 50 µL of standard to 85 µL of diluent #1, mix gently and repeat the 1/2.7 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 #1 or appropriate culture medium alone



STANDARD	SERIAL DILUTIONS	WORKING SOLUTIONS
Standard Stock solution	Thaw frozen stock solution	40 000 ng/mL
Standard 7	10 µL Standard stock Solution + 90 µL diluent	4 000 ng/mL
Standard 6	50 µL Standard 7 + 85 µ L diluent	1 481 ng/mL
Standard 5	50 µL standard 6 + 85 µL diluent	549 ng/mL
Standard 4	50 µL standard 5 + 85 µL diluent	203 ng/mL
Standard 3	50 µL standard 4 + 85 µL diluent	75.3 ng/mL
Standard 2	50 µL standard 3 + 85 µL diluent	27.9 ng/mL
Standard 1	50 µL standard 2 + 85 µL diluent	10.3 ng/mL
Standard 0	85 µL diluent	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 in your appropriate sample medium.
- To obtain additional information or support, please contact the HTRF technical support team.

ASSAY PROTOCOL

		STANDARD (STD 0 – STD 7)	SAMPLES
Step 1		Dispense 5 µL of each ApoB standard (Std 0 - Std 6) into each standard well	Dispense 5 µL of each sample into each sample well
Step 2		Add 5 µL of diluent	
Step 3		Add 10 µL of detection reagent mix) to all wells (ApoB Eu Cryptate antibody & ApoB d2 antibody working solutions mixed 1:1).	
Step 4		Seal the plate and incubate ON at RT	
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) 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well A1	Repeat Well A1	5 µL sample 1 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well A4	Repeat Well A4
B	5 µL Std 1 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well B1	Repeat Well B1	5 µL sample 2 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well B4	Repeat Well B4
C	5 µL Std 2 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well C1	Repeat Well C1	5 µL sample 3 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well C4	Repeat Well C4
D	5 µL Std 3 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well D1	Repeat Well D1	5 µL sample... 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well D4	Repeat Well D4
E	5 µL Std 4 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well E1	Repeat Well E1	5 µL sample... 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well E4	Repeat Well E4
F	5 µL Std 5 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well F1	Repeat Well F1	5 µL sample... 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well F4	Repeat Well F4
G	5 µL Std 6 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well G1	Repeat Well G1	5 µL sample... 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well G4	Repeat Well G4
H	5 µL Std 7 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well H1	Repeat Well H1	5 µL sample... 5µL of diluent 5 µL ApoB-d2 5 µL ApoB-Eu Cryptate	Repeat Well H4	Repeat Well H4

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
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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.

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

3) Calculate specific signal for each triplicate.

$$\text{Delta ratio} = \text{Positive ratio (for each standard)} - \text{Negative ratio}$$

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

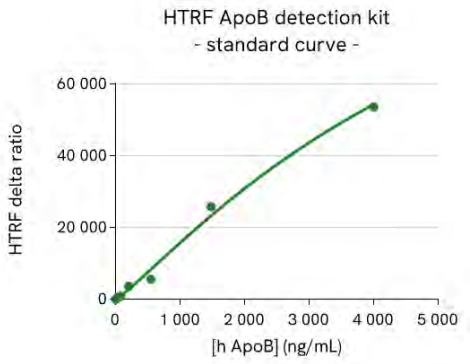
RESULTS

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 (3)	CV% (2)
Standard 0	Negative control	970	0	2%
Standard 1	10.3 ng/mL	1191	216	1%
Standard 2	27.9 ng/mL	1465	489	5%
Standard 3	75.3 ng/mL	1815	840	11%
Standard 4	203 ng/mL	4359	3384	10%
Standard 5	549 ng/mL	6534	5559	3%
Standard 6	1481 ng/mL	26321	25346	4%
Standard 7	4 000 ng/mL	53462	52486	4%



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

www.revvity.com

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

(800) 762-4000
www.revvity.com

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