

Human Apolipoprotein B kit 500 tests

For in vitro research use only

Storage temperature : -60°C or below

Packaging details :

64APBPEG	384-well low volume plate (20 µL)
	500 tests

Product information:

Document reference : 64APBPEG - Rev 05 - May 2021

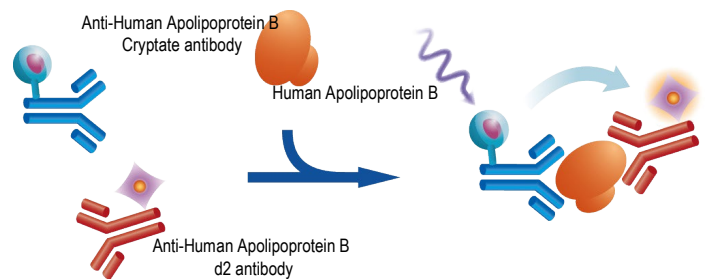
1. ASSAY DESCRIPTION AND INTENDED USE

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

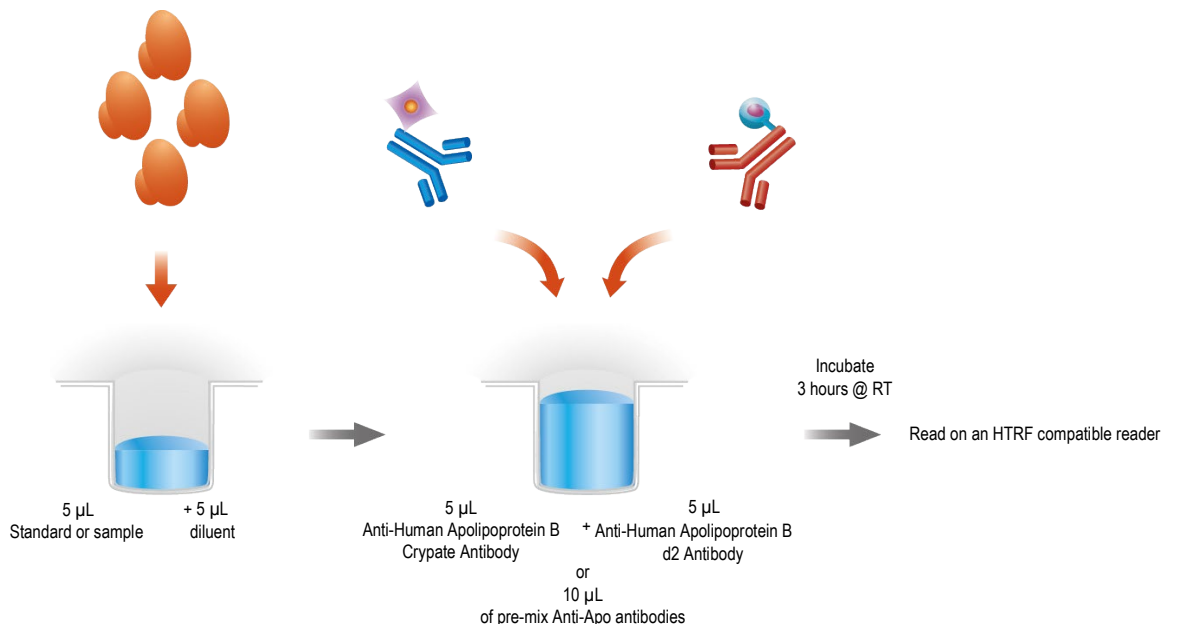
As shown in the diagram to the right, Human Apolipoprotein B is detected in a sandwich assay format using two different specific antibodies, one labeled with Eu³⁺-Cryptate (donor) and the second with d2 (acceptor).

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).





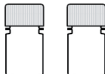
The two antibodies bind to the antigen present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the Human Apolipoprotein B concentration.



2. PROTOCOL AT GLANCE



3. HTRF REAGENTS

	Human Apo-B Standard	Anti-Human Apo-B d2 antibody	Anti-Human Apo-B Eu ³⁺ -Cryptate-antibody	Diluent	Detection buffer #3
					
Stock solution	200 µL/vial 10 µg/mL	50 µL/vial	50 µL/vial	20 mL/vial	7 mL/vial, 2 vials
Storage	-60°C or below	-60°C or below	-60°C or below	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-B 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-B solutions with the same precautions as potentially infectious specimens.

4. 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.

5. 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 B-d2 antibody to 1 volume of anti-Human Apolipoprotein B-Eu³⁺ cryptate antibody, and dispense 10 µL of the pre-mix anti-Human Apolipoprotein B 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 more information about HTRF compatible readers, please visit our website at: <http://www.cisbio.com/readers>

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

5.1. Preparation of antibody working solutions

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

Anti-Human Apolipoprotein B-d2 antibody	Anti-Human Apolipoprotein B-Eu ³⁺ -Cryptate-antibody
Dilute 50-fold the stock solution of anti-human Apo-B-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-B-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.

5.2. 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

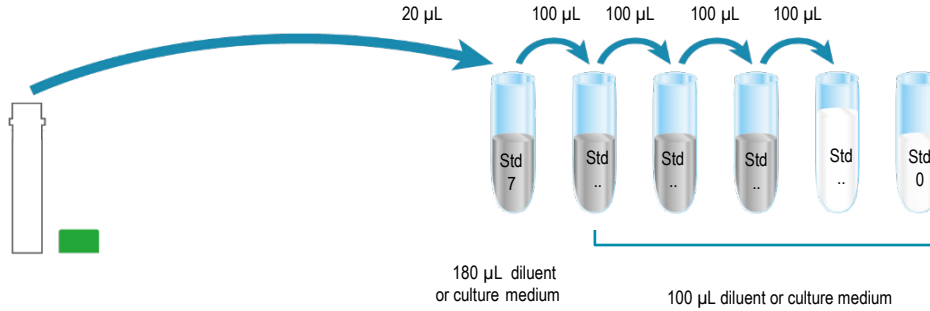
Standards	Working concentration (ng/mL)	Preparation
Std 7	1,000	200 µL standard stock solution
Std 6	500	100 µL Std 7 + 100 µL diluent or culture medium
Std 5	250	100 µL Std 6 + 100 µL diluent or culture medium
Std 4	125	100 µL Std 5 + 100 µL diluent or culture medium
Std 3	62.5	100 µL Std 4 + 100 µL diluent or culture medium
Std 2	31.25	100 µL Std 3 + 100 µL diluent or culture medium
Std 1	15.6	100 µL Std 2 + 100 µL diluent or culture medium
Std 0	0	100 µL diluent or culture medium

A recommended standard dilution procedure is listed and illustrated after:

1. Pre-dilute the standard stock solution 10-fold with diluent. In practice: take 10 μ L of stock solution and add 90 μ L of diluent or culture medium in the standard stock solution. Mix gently. This yields the high standard (Std 7: 1,000 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 μ L of diluent in each vial from Std 6 to Std 1.
 - Add 100 μ L of standard to 100 μ L of diluent, mix gently and repeat the 1/2 serial dilution to make standard solutions: 500, 250 125, 62.5, 31.25, 15.6 ng/mL.

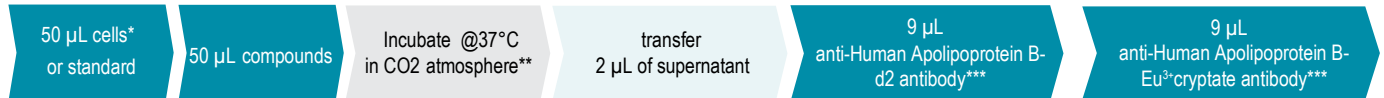
This will create 7 standards for the analyte. Std 0 (Negative control) is diluent or culture medium alone.

The standard dilution procedure is listed and illustrated below.



6. EXTENDED DYNAMIC RANGE PROTOCOL: SUPERNATANT

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 B-d2 antibody to 1 volume of anti-Human Apolipoprotein B-Eu³⁺ cryptate antibody, and dispense 18 μ L of the pre-mix anti-Human Apolipoprotein B antibodies.

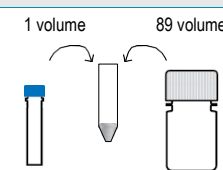
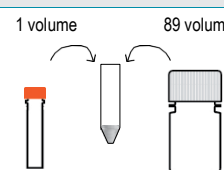
- Cover the plate with a plate sealer.
- Incubate at RT for 3 hours.
- Remove the plate sealer and,
- Read the fluorescence emission at two different wavelengths (665nm and 620nm) on a compatible HTRF[®] reader.

For more information about HTRF compatible readers, please visit our website at: <http://www.cisbio.com/readers>

	Assay controls			Supernatant / Std
	Negative control	Cryptate control	Buffer control	
	Used to calculate the delta F %	Used to check the Cryptate signal at 620nm	used to check background fluorescence	
Supernatant / Std	-	-	-	2 μ L
Diluent	2 μ L	2 μ L	2 μ L	-
Anti-Human Apolipoprotein B-d2 antibody	9 μ L	-	-	9 μ L
Anti-Human Apolipoprotein B-Eu ³⁺ -Cryptate antibody	9 μ L	9 μ L	-	9 μ L
Detection buffer #3	-	9 μ L	18 μ L	-

6.1. Preparation of antibody working solutions

Determine the amount of antibody needed for the experiment. Each well requires 9 μL of each antibody.

Anti-Human Apolipoprotein B-d2 antibody	Anti-Human Apolipoprotein B-Eu ³⁺ -Cryptate-antibody
	
Dilute 90-fold the stock solution of anti-human Apo-B-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-B-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.

6.2. Standard curve preparation

Determine how many samples and replicates will be tested.

Each well requires 2 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

Standards	Working concentration (ng/mL)	Preparation
Std 7	4,000	200 μL standard stock solution
Std 6	2,000	100 μL Std 7 + 100 μL diluent or culture medium
Std 5	1,000	100 μL Std 6 + 100 μL diluent or culture medium
Std 4	500	100 μL Std 5 + 100 μL diluent or culture medium
Std 3	250	100 μL Std 4 + 100 μL diluent or culture medium
Std 2	125	100 μL Std 3 + 100 μL diluent or culture medium
Std 1	62.5	100 μL Std 2 + 100 μL diluent or culture medium
Std 0	0	100 μL diluent or culture medium

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 40 μL 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.

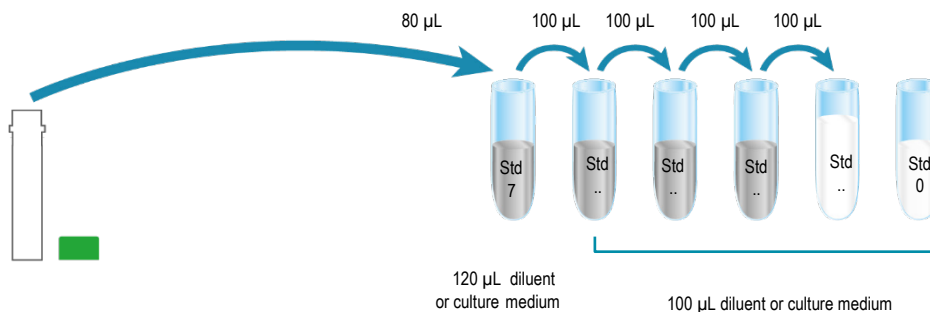
2. Prepare the following serial dilutions:

- 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 100 μL of standard to 100 μL 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.



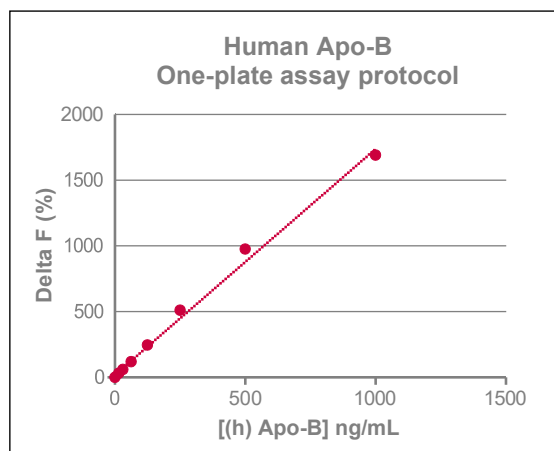
7. DATA REDUCTION

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.

The assay standard curve is created by plotting delta F% versus the analyte concentration:

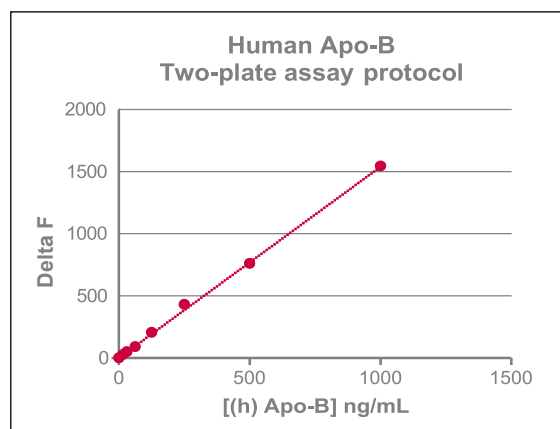
One-plate assay protocol

[Apo-B Std] (ng/mL)	Ratio ⁽¹⁾	CV % ⁽²⁾	Delta F % ⁽³⁾
0	513	1%	0%
15.6	667	2%	30%
31.25	819	1%	60%
62.5	1125	2%	119%
125	1776	1%	246%
250	3126	1%	510%
500	5512	2%	975%
1,000	9183	2%	1691%



Two-plate assay protocol

[Apo-B Std] (ng/mL)	Ratio ⁽¹⁾	CV % ⁽²⁾	Delta F % ⁽³⁾
0	490	2%	0%
15.6	627	3%	28%
31.25	740	0%	51%
62.5	939	2%	92%
125	1511	1%	208%
250	2607	4%	432%
500	4227	1%	763%
1,000	8056	2%	1545%



To obtain additional information or support, please contact the HTRF technical support team at htrfservices@cisbio.com

8. Analytical characteristics

8.1 Standardization

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

8.2 Cross-reactivity

	Cross-reactivity %
Apo-B100	100%
Apo-A1	0.004%
Apo-A2	< 0.001%
Apo-C1	< 0.001%
Apo-C2	0.001%
Apo-C3	0.006%
Apo-E	0.084%

8.3. Detection limit

One-plate assay protocol: 0.8 ng/mL

Two-plate assay protocol (supernatant): 9 ng/mL

8.4. Extended range

One-plate assay protocol: 1,000 ng/mL

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