ab204717

HDL Uptake Assay Kit (Fluorometric)

Instructions for Use

For rapid, sensitive and accurate measuring of HDL uptake.

This product is for research use only and is not intended for diagnostic use.

Version 2e Last Updated 16 November 2020

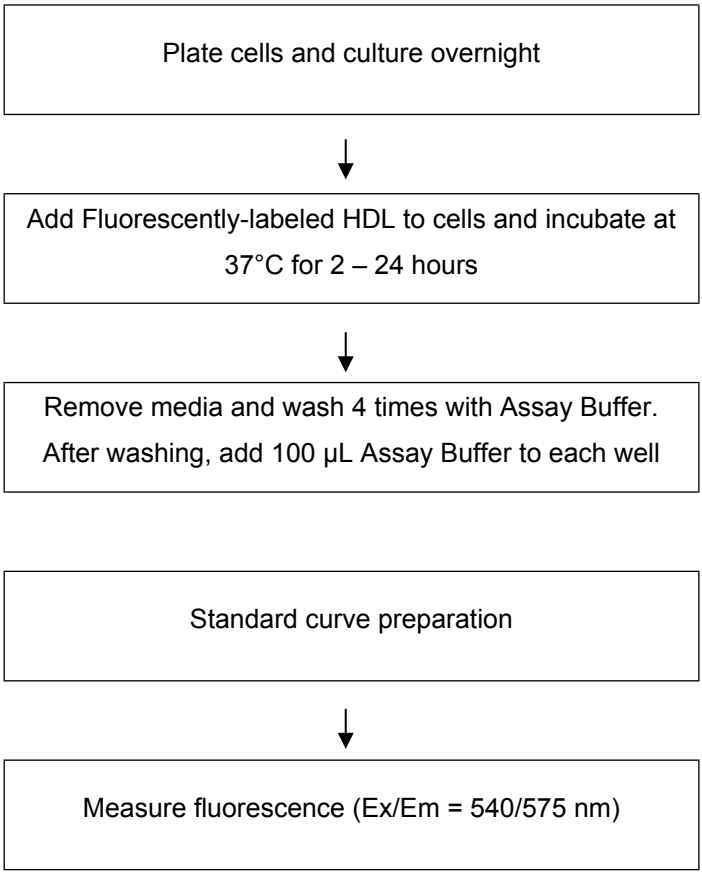
Table of Contents

1. BACKGROUND

HDL Uptake Assay Kit (Fluorometric) (ab204717) contains a fluorescently-labeled HDL that can be measured when taken up by cells. Unlabeled-HDL is included in the kit for assay validation. HDLs used for labelling are from humans.

High-density lipoprotein (HDL) consists of a protein shell, containing apolipoproteins A-I and A-II, and a hydrophobic core consisting of cholesterol and triglycerides surrounded by phospholipids and cholesterol esters. HDL transports cholesterol to the liver or steroidogenic organs such as adrenals, ovary, and testes by both direct and indirect pathways. HDL-Cholesterol is removed by HDL receptors such as scavenger receptor BI (SR-BI), which mediate the selective uptake of cholesterol from HDL. Clinically, high levels of HDL has been associated with cardiovascular health. This is due to the ability of HDL particle to transport cholesterol from lipid-laden macrophages of atherosclerotic arteries to the liver for secretion into the bile by a process called as reverse cholesterol transport.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been opened.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Opened components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer	100 mL	-20°C	-20°C
Fluorescently-labeled HDL (5 mg/mL)	1 mL	-20°C	-20°C
Unlabeled HDL	100 µL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

These materials are not included in the kit, but will be required to successfully perform this assay:

- CO_2 incubator (37°C), tissue culture plasticware
- Complete growth medium
- Fetal Bovine Serum (FBS)
- Centrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter for Ex/Em = 540/575 nm
- 96 well plate: white with clear flat bottom
- Syringe and 0.22 µm syringe filter

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Fluorescently-labeled HDL (5 mg/mL):**

Thaw on ice. Fluorescently-labeled HDL can be stored at 4°C for 1 week. For long term storage, aliquot labeled HDL so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep protected from light and on ice while in use.

9.3 **Unlabeled HDL (2X):**

Thaw on ice. Unlabeled HDL can be stored at 4°C for 1 week. For long term storage, aliquot unlabeled HDL so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep protected from light and on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and cannot be stored for future usage.

10.1 Prepare a 1:100 dilution of the fluorescently-labeled HDL by adding 5 µL of labeled HDL into 495 µL of Assay Buffer

10.2 Using the diluted Fluorescently-labeled HDL standard, prepare standard curve serial dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Sample to Dilute	Volume to dilute (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Conc. in well (ng/well)
1	Stock	30	570	100	250
2	Standard #1	300	300	100	125
3	Standard #2	300	300	100	62.5
4	Standard #3	300	300	100	31.2
5	Standard #4	300	300	100	15.6
6	Standard #5	300	300	100	7.8
7	Standard #6	300	300	100	3.9
8	0		300	100	0

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 µL).

NOTE: *Standard already contains fluorescently-labeled HDL so it doesn't need additional reagents. Standard can be prepared during sample incubation with labeled-LDL (Step 12.5).*

11. SAMPLE PREPARATION

General Sample information:

- Cells should be maintained via standard tissue culture practices. Always make sure that cells are healthy and in the log phase of growth before using them for an experiment.

11.1 Cell (adherent or suspension) samples:

11.1.1 Plate 3 – 4 x 10^4 cells/well in a 96-well white clear-bottom cell culture plate and culture in media with serum.

11.1.2 Incubate cells overnight at 37°C in a CO_2 incubator.

11.1.3 OPTIONAL: To increase the LDL uptake, cells can be starved in serum-free media for 4 – 16 hours after overnight incubation step.

11.1.4 Remove media and wash cells 3 times with 100 µL Assay Buffer.

NOTE: *For suspension cells, spin 96-well plate at 1,000 x g for 5 minutes before every media/buffer exchange.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Sample wells = see Sample Preparation section.
- Background control sample wells= add 100 µL serum-free media (do not add reaction mix).
- Specificity HDL uptake control well = 10 µL Unlabeled HDL (to compete with the fluorescently-labeled HDL signal).

12.2 **Reaction Mix:**

Prepare 100 µL of diluted Fluorescently-labeled HDL for each well:

Component	Substrate Solution (µL)
Fluorescently-labeled HDL	**2**
Serum-free media	**98**

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions + 2).

NOTE: *Concentration of the Fluorescently-labeled HDL may vary for different cell types. We recommend using 2 µL – 10 µL (~10 µg – 50 µg) of Fluorescently-labeled HDL depending on the cell type.*

12.3 Sterilize Reaction Mix using a 0.22 µm syringe filter.

12.4 Add 100 µL Reaction Mix to each sample and specificity control well.

12.5 Incubate at 37°C for 2 – 24 hours or as desired.

If using stimulants/inhibitors of HDL uptake, pre-incubate with cells 1 – 2 hours before and during treatment with fluorescently-labeled HDL.

12.6 Remove media and carefully wash 4 times with 100 µL Assay Buffer.

NOTE: *For suspension cells, spin 96-well plate at 1,000 x g for 5 minutes before every media/buffer exchange*

12.7 Add 100 µL Assay Buffer to each well.

12.8 Set up Standard wells by adding 100 µL of standard (from Step 10.1) to empty wells in the microplate.

12.9 Protect from light. Measure fluorescence in a microplate reader at Ex/Em = 540/575 nm.

NOTE: *To determine the non-specific Fluorescently-labeled HDL binding, perform a wash off step. Incubate cells with diluted Fluorescently-labeled HDL for 2 minutes. Remove media containing Fluorescently-labeled HDL and wash 4 times with 100 µL of Assay Buffer. Add 100 µL of Assay Buffer and measure.*

13. CALCULATIONS

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean RFU value of the blank (Standard #8) from all standard and sample readings. This is the corrected RFU.

13.4 Plot the corrected RFU values for each standard as a function of the final concentration of HDL.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Concentration of HDL µg/mg protein/hr in the test samples is calculated as:

$$HDL\ Uptake = \left(\frac{B}{P * T}\right)$$

Where:

B = Amount of Fluorescently-labeled HDL in the sample well from Standard Curve (µg).

P = Protein concentration (mg).

T = Incubation time (hours).

To measure protein concentration, lyse cells in 100 µL cell lysis buffer and measure protein concentration using a BCA protein assay.

NOTE: *LDL uptake can also be expressed as µg/number of cells [(10^4)/hr].*

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

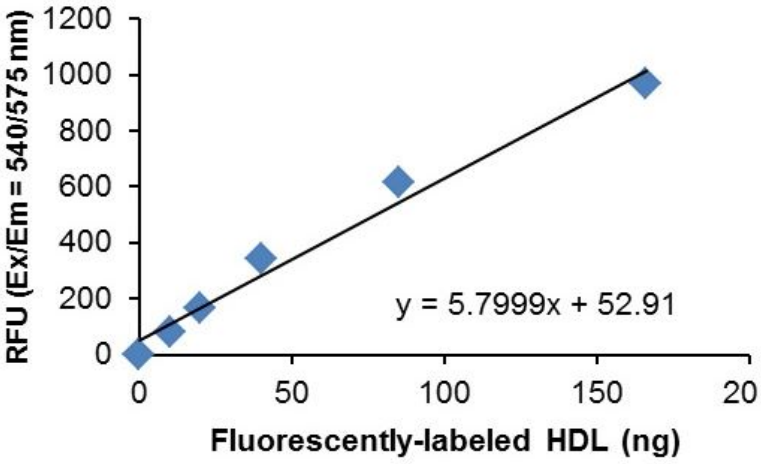


Figure 1. Fluorescently-labeled HDL Standard curve.

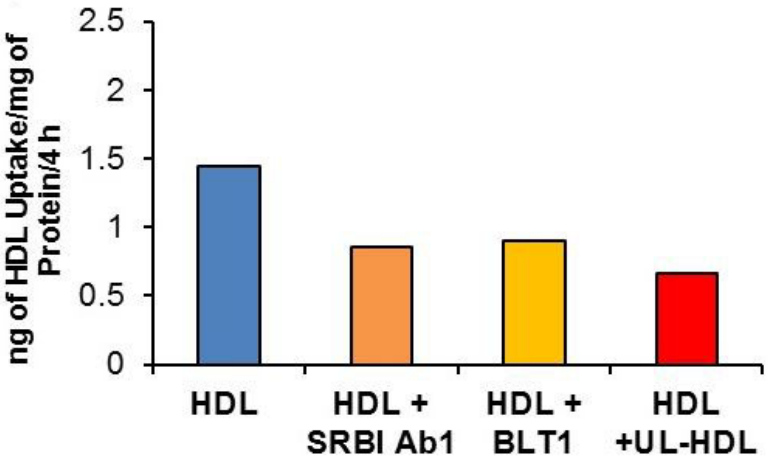


Figure 2. Fluorescently-labeled HDL uptake in HepG2 (hepatocytes) cells. Cells were starved for 4 hours and incubated with Fluorescently-labeled HDL (10 µg) for 4 hours. Fluorescently-labeled HDL (HDL) uptake was inhibited by SR-BI (HDL receptor) antibody (HDL + SRB1 Ab1), BLT1 (SR-BI Inhibitor; HDL + BLT1) and outcompeted by Unlabeled-HDL (HDL+ UL-HDL [20 µg]). Background was subtracted from the data (Wash off as described in Section 12 note c, was subtracted as background). Assay was performed following the kit protocol.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Plate 3-4 x 10^4 cells/well in a 96-well white clear-bottom cell culture plate and culture in media with serum overnight.
- Remove media and wash 3 times with Assay Buffer.
- Set up background control (100 µL serum-free media) and HDL uptake specificity well (100 µL diluted Fluorescently-labeled HDL + 10 µL Unlabeled HDL).
- Prepare diluted Fluorescently-labeled HDL.

Component	Solution (µL)
Fluorescently-labeled HDL	**2**
Serum-free media	**98**

- Add 100 µL of diluted Fluorescently-labeled HDL to sample and control wells.
- Incubate at 37°C for 2-24 hours.
- Prepare standard curve.
- Remove media and carefully wash 4 times with Assay Buffer.
- Add 100 µL Assay Buffer to each well.
- Set up Standard wells (100 µL).
- Measure fluorescence (Ex/Em = 540/575 nm)

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)