ab196985 – Cholesterol Efflux Assay Kit (Cell-based)

For the quantative measurement of cholesterol efflux.
For research use only - not intended for diagnostic use.
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab196985

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 1 year, provided components have not been reconstituted. Reconstituted components are stable for 2 months.

Materials Supplied

Item	Quantity	Storage Condition
Lysis Buffer I/Cell Lysis Buffer	25 mL	-20°C
Equilibration Buffer	5 mL	-20°C
Labeling Reagent/Labelling Reagent	5 mL	-20°C
Cholesterol Uptake Positive Control/Positive Control	1 mL	-20°C
Reagent A	10 µl	-20°C
Reagent B	50 µl	-20°C
Serum Treatment Reagent	1 mL	-20°C

Materials Required, Not Supplied

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

- Macrophage cell line (e.g. J774A.1 cells).
- Phenol red-free RPMI-1640 culture medium, Fetal Bovine Serum (FBS) and 5% CO2 cell culture incubator.
- Multiwell fluorescence microplate reader.
- Multi-channel pipette.
- Clear 96-well tissue-culture plate (one) and white 96-well plate with opaque flat-bottom wells (two).

Reagent Preparation

- Open all the reagents under sterile conditions (e.g. a cell culture hood) only.

Labeling Reagent/Labelling Reagent: Store at -20°C, protected from light. Thaw to room temperature before use and open under sterile conditions.

Equilibration Buffer: Store at -20°C, protected from light. For each experiment, aliquot required amount of equilibration buffer for the experiment. Immediately prior to use, add Reagent A (2 µl/ml) and Reagent B (10 µl/ml) to aliquoted equilibration buffer under sterile conditions and warm to 37°C before adding to the cells.

Lysis Buffer I/Cell Lysis Buffer: Store at -20°C.Warm to room temperature before adding to the cells.

Cholesterol Uptake Positive Control/Positive Control: Store at -20°C. Thaw at room temperature and warm to 37°C before adding to the cells.

Serum Treatment Reagent: Store at -20°C. Thaw at room temperature prior to use.

Cholesterol Efflux Assay Protocol

The procedure described below is for murine macrophage cell line J774.1. This procedure can also be used with macrophage cells derived from THP-1 monocytes, which can be differentiated into cells with a macrophage-like phenotype upon treatment with 100 nM phorbol 12-myristate-13-acetate (PMA) for 48-72 hours.

Load Cells with Fluorescently-Labelled Cholesterol:

1. Grow J774.1 macrophage cells in growth medium (RPMI 1640 with 10% FBS) until cells reach ~90% confluency. Split cells under sterile conditions (do not use trypsin for detachment when splitting/seeding cells, see note below) and seed approximately 1 x 105 J774.1 cells/well in a 96-well tissue culture plate using 100 µl growth medium per well.
2. When planning the assay, remember to seed additional wells for assay validation (positive and negative (no cholesterol acceptor) control conditions).
3. Incubate cells for 4-6 hours at 37°C (in a humidified cell culture incubator with a 5% CO_2 atmosphere) to allow cells to fully adhere to the plate.

Δ Notes:

a) Treatment of macrophage (J774.1 or THP-1) cells with trypsin or other cell dissociation solutions can destroy the extracellular matrix scaffolding required for cholesterol acceptor binding and will inhibit or ablate cholesterol efflux. We recommend using manual scraping with a rubber cell scraper to split and seed macrophage cultures.

b) To ensure accuracy, we recommend each planned treatment condition (including each test cholesterol acceptor, positive control and negative (no cholesterol acceptor) controls) to be performed in duplicate or triplicate wells.

4. For each test well (including positive and negative cholesterol acceptor control wells), prepare 100 µl of labelling medium by mixing 50 µl of Labeling Reagent/Labelling Reagent and 50 µl of serum-free RPMI medium.
5. Gently aspirate growth medium from wells and wash the cell monolayer with serum-free RPMI medium. Add 100 µl of the labelling medium mix per well. Incubate the plate for 1 hour, protected from light at 37°C in a humidified incubator with a 5% CO2 atmosphere.
6. Following labelling, prepare 100 µl of equilibration medium for each well by mixing 50 µl Equilibration Buffer (with appropriate concentrations of Reagent A and B added immediately before use, see Section VI) and 50 µl of serum-free RPMI medium.
7. Aspirate labelling medium from wells and add 100 µl of the equilibration medium mix per well.
8. Incubate the plate overnight (12-16 hours), protected from light at 37°C in a humidified incubator with a 5% CO2 atmosphere.

Treat Cells with Cholesterol Acceptor:

1. If using human serum as a cholesterol acceptor, prepare LDL/VLDL-depleted serum using the Serum Treatment Reagent prior to addition to cells.
2. Add 2 parts of Serum Treatment Reagent to 5 parts of human serum (a 2:5 v/v ratio; for example, mix 40 µl Serum Treatment Reagent with 100 µl human serum).
3. Incubate mixture for 20 min on ice.
4. Centrifuge the mixture at 9000 x g for 10 min at 4°C and transfer supernatant to a clean microfuge tube. Keep on ice until used.
5. After overnight incubation, gently aspirate the equilibration medium from cells and wash by adding 200 µl of phenol red-free, serum-free RPMI medium to each well.
6. Aspirate the wash medium and add desired cholesterol acceptors diluted in phenol red-free, serum-free RPMI medium, according to the table below. If using pre-treated human serum (prepared above), we recommend using 2 µl supernatant per well (2% of the total well volume). However, 1-4 µl of LDL/VLDL-depleted serum (1-4% of the total well volume) may be used if desired.

7. For cholesterol acceptor positive control, add 20 µl of Cholesterol Uptake Positive Control/Positive Control and bring the volume up to 100 µl with serum-free RPMI. For negative control (no cholesterol acceptor), add 100 µl serum-free RPMI to negative control well(s).

	Pre-treated Serum	Purified HDL	Cholesterol Uptake Positive Control/Positive Control	Negative Control
Sample	2 µl	2.5 – 20 µg	20 µl	-
Serum-Free RMPI-1640	To 100 µl	To 100 µl	80 µl	100 µl

Δ Notes:

a) If desired, test compounds (i.e. potential stimulants or inhibitors of cholesterol efflux) may be added during the efflux incubation period. Test compound stock solutions should be diluted in phenol red-free, serum-free RPMI to 10X desired final concentration.
b) For measurement of cholesterol efflux to a given acceptor in the presence of test compounds, bring the well volume up to 90 µl with serum-free RMPI (instead of 100 µl) and add 10 µl of the 10X test compound solution.
c) If an organic solvent is used to dissolve test compound, we recommend performing a vehicle condition (with the same final concentration of solvent) to control for solvent effects.

8. Return plate to 5% CO2 incubator and incubate cells treated with cholesterol acceptors at 37°C for 4 hours (or desired time based upon your specific treatment conditions and protocol), protected from light.

Measurement

1. At the end of incubation, transfer supernatant (medium containing cholesterol acceptor) of each well (including control wells) to a white 96-well plate (with opaque, flat-bottom wells) and measure the fluorescence (Ex/Em = 485/523 nm) in endpoint mode.
2. Solubilize the adherent cell monolayer by adding 100 µl of Lysis Buffer I/Cell Lysis Buffer to each well and incubating the plate (protected from light) with orbital shaking at room temperature for 30 min.
3. Pipette up and down to dissolve any cell debris and transfer cell lysate to another white 96-well plate (with opaque, flat-bottom wells) of the same type used for the supernatant.
4. Measure the fluorescence (Ex/Em = 485/523 nm) of the cell lysate.

Calculation

1. Cholesterol efflux from the labeled macrophage cells to a particular cholesterol acceptor/treatment condition is calculated by dividing the fluorescence intensity (RFU) obtained for the supernatant by the sum of the fluorescence intensity of the supernatant and cell lysate of the same treatment.
2. This value is multiplied by 100 to obtain %Cholesterol Efflux in 4 hours.
3. Subtract %Cholesterol Efflux obtained from the negative (no treatment) control from the treatment groups to determine the final net %Cholesterol Efflux.

$$\% \text{ Cholesterol Efflux} = \frac{RFU\ of\ Supernatant}{RFU\ of\ Cell\ Lysate\ +\ RFU\ of\ Supernatant} \times 100$$

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)