Cholesterol Efflux Fluorometric Assay Kit (Cell-Based) (BN00811)

(Catalog # BN00811; 100 assays; Store at -20°C)

I. Introduction:

Cholesterol efflux from the peripheral tissues and cells in atherosclerotic plaque is an initial and critical step in Reverse Cholesterol Transport (RCT). RCT is the process by which extrahepatic cells, including macrophage-derived foam cells in arterial atherosclerotic plaque, transport excessive cholesterol back to the liver for bile acid synthesis and excretion, thus lowering the peripheral lipid burden. A negative correlation has been established between the in vitro efflux of cholesterol from macrophages and atherosclerosis. Assay Genie's Cholesterol Efflux Assay is a high-throughput screening assay for measuring cholesterol efflux in cells using fluorescently-labeled cholesterol. This assay provides a safe, sensitive, and reproducible method for measuring cholesterol efflux.

II. Application:

- Screen serum samples or lipoproteins (isolated or recombinant) for cholesterol efflux.
- Screen small molecules for their effect on cholesterol efflux (a valuable tool for drug discovery program).

III. Sample Type:

Serum, Isolated, or recombinant lipoproteins

IV. Kit Contents:

Components	BN00811	Cap Code	Part Number
Labeling Reagent	5 ml	Amber	BN00811-1
Equilibration Buffer	5 ml	NM	BN00811-2
Reagent A	10 µl	Green	BN00811-3
Reagent B	10 µl	Yellow	BN00811-4
Cell Lysis Buffer	20 ml	WM	BN00811-5
Positive Control	1 ml	Blue	BN00811-6
Serum Treatment Reagent	1 ml	Purple	BN00811-7

V. User Supplied Reagents & Equipments:

- J774A.1 Macrophage cell line.
- Media to grow cells (RPMI 1640 and Fetal Bovine Serum (FBS)).
- Spectrophotometer capable of reading fluorescence.
- Multi-channel pipette.
- 96-well white plate with clear bottom (one) and 96-well white plate (one).

VI. Storage and Handling:

Store kit at -20°C, protected from light. Read the entire protocol before performing the assay. Open all the reagents under sterile conditions (e.g. cell culture hood).

VII. Reagent Preparation and Storage Conditions:

- **Labeling Reagent:** Store at -20°C. Protect from light. Thaw on ice before adding to the cells.
- **Equilibration Media:** Store at -20°C. Warm at 37°C before use. Aliquot required amount of equilibration media for the experiment. Add Reagent A 2 µl/ml and Reagent B 2 µl/ml to the media just before use under sterile conditions.
- **Cell Lysis Buffer:** Store at -20°C.Thaw before adding to the cells.
- **Positive Control:** Store at -20°C. Thaw on ice before adding to the cells.
- **Serum Treatment Reagent:** Store at -20°C. Thaw before adding to the cells.

VIII. Cholesterol Efflux Assay Protocol:

The procedure described below is for macrophage cell line J774.1. This procedure can also be used with macrophage cells derived from THP-1 monocytes, by addition of 100 nM phorbol 12-myristate 13-acetate (PMA) for 48 hrs.

1. **Label Cells:** Grow J774.1 cells in RPMI 1640 media containing 10% FBS in cell culture flask till ~90% confluency (37°C incubator containing 5% CO_2). Split cells under sterile conditions using basic cell culture techniques & plate approximately 1 x 10^5 J774.1 cells/well in a 96-well plate (white plate with clear bottom) using 100 µl media/well. Grow for 2 hrs. in a 37°C incubator containing 5% CO_2. After 2 hrs., when the cells are attached to the plates, wash the cell monolayer with RPMI 1640 media (no serum added). Premix 50 µl of Labeling Reagent and 50 µl of Equilibration Buffer containing Reagent A and B/well just before use. Add 100 µl mix/well. Incubate the plate overnight (16 hrs.) in a 37°C incubator containing 5% CO_2.

   Notes:

   a. To test the background fluorescence, add 100 µl of Equilibration Buffer containing Reagent A and B to the cell monolayer. Don't add labelling reagent to the background control well(s).

   b. For accurate assay, we recommend each treatment and control to be performed in triplicates.

2. **Treat Cells:** After 16 hrs., remove the Labeling Reagent. Wash the cells gently by adding 200 µl of RPMI media (no serum) to all the wells. Remove the media. Treat cells with desired cholesterol acceptors in RPMI media. If using human serum as cholesterol acceptor,

pre-treat the serum with Serum Treatment Reagent. Add 2 parts of Serum Treatment Reagent to 5 parts of human serum (Ratio 2:5). Incubate for 20 min. on ice. Centrifuge the mixture at 9,000 x g, for 10 min. at 4°C. Use the supernatant to treat the cells as desired and make up the volume to 100 µl by RPMI media. For Positive Control, add 20 µl of Positive Control & make up the volume to 100 µl by RPMI media. For no treatment control, add 100 µl RPMI media (no serum) to no treatment control well(s). Incubate cells for 4-6 hrs. in a 37°C incubator containing 5% CO_2.

3. **Measurement**: At the end of incubation, transfer supernatant to a 96-well plate (white plate). Measure the fluorescence (Ex/Em = 482/515 nm). Solubilize the cell monolayer by adding 100 µl of Cell Lysis Buffer and shaking on a plate shaker for 30 min. at room temperature. Pipette up and down to dissolve any cell debris. Measure the fluorescence (Ex/Em = 482/515 nm).
4. **Calculations**: Cholesterol efflux of the treatments is calculated by dividing the fluorescence intensity of the media by total fluorescence intensity of the cell lysate of the same treatment & media. This value is multiplied by 100 to obtain % Cholesterol Efflux. Subtract % Cholesterol Efflux obtained from no treatment control from the treatment groups to determine the final % Cholesterol Efflux.

$$\text{\% Cholesterol Efflux} = \frac{\text{Fluorescence intensity of the media}}{\text{Fluorescence intensity of the cell lysate + media}} \text{ X 100}$$

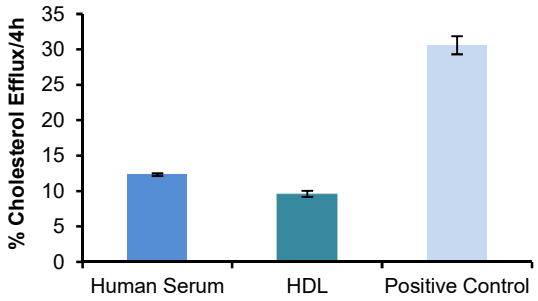

Figure: Percentage (%) Cholesterol Efflux: J774.1 cells were labeled with the Labeling Media and treated with various cholesterol acceptors like Human Serum, HDL (50 µg) or Positive control known to cause cholesterol efflux. Cholesterol efflux is expressed as % efflux elicited by cells in 4h.

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