**A High-throughput, Fluorescence-based Cholesterol Efflux Assay**

Sonika Saddar, Tasnim Kothambawala, Nick Chacos, Preeti Saini, Grigoriy Tchaga and Gordon Yan

BioVision Inc., Milpitas, California 95035

sonikas@biovision.com

---

### Introduction

Numerous studies have established a negative correlation between cellular cholesterol efflux and cardiovascular disease. Cholesterol efflux from peripheral tissues and cells by a process called Reverse Cholesterol Transport (RCT). RCT allows egress of cholesterol from the arterial wall and its return to the liver, thus lowering the peripheral lipid burden. Standard protocols for quantitation of cholesterol efflux involve labeling cells with tritiated cholesterol and measuring the release of labeled sterol in a 12 or 24 well plate. This protocol, however, is not ideal for high-throughput screening of large numbers of serum samples or screening compounds/small molecules for their effect on cholesterol efflux. We report here a simple, convenient and high-throughput cell-based assay using fluorescently-labeled cholesterol in a 96-well plate format. Our results demonstrate that the percentage cholesterol efflux elicited by 2% ApoB-depleted normal human serum in J774 macrophage cells is comparable to those published in literature. Our assay utilizes a positive control for this assay, which induces efflux independent of any receptor, that is used as a measure of the reliability of the assay. This assay is highly reproducible with low inter-assay variability (±10%). We demonstrate that this is an efficient method of measuring cholesterol efflux in a cell-based assay. The increased sensitivity of the assay coupled with its high-throughput screening capability enables scientists to screen large libraries of reagents and serum samples and provides a valuable tool for drug discovery in the field of cardiovascular disease research.

### Key Features

**Macrophage Cell Line**

- Cell Plating/Culture

**Labeled Cholesterol + Reagent A + Reagent B**

- 2% ApoB-depleted Serum
- Cholesterol Efflux Capacity

**Simple & Rapid Protocol**

- Convenient: Non-Radioactive, no special handling or disposal required
- High-Throughput

- Extremely Stable: long shelf life

- Accurate: reproducible results with low intra & inter assay variability

- Fluorometric Quantitation (Ex/Em = 482/515 nm)

- Ample reagents to perform 100 assays in 96-well plate format

---

### An in-vitro Assay to Measure Cholesterol Efflux

**A.**

- **Liver**
  - **Effluxed Cholesterol**

**B.**

- **Extracellular Space**
  - **ABCA1**
  - **SRBI**
  - **PON1**
  - **Cyp7A1**
  - **Caveolin**

**Macrophage**

**Cholesterol Efflux 4h**

### Multiple Applications of in-vitro Cholesterol Efflux Assay

- Screen serum samples or lipoprotein (isolated or recombinant) for cholesterol efflux.
- Screen small molecules for their effect on cholesterol efflux (a valuable tool for drug discovery program).
- Measure cholesterol efflux using variety of primary cells or cell lines to understand the role of different receptors or acceptor molecules in cholesterol efflux (Figure 2).
- Screen receptor-specific inhibitor(s) for cholesterol efflux (Figure 2 B).

---

### Cholesterol Efflux from Patients with Coronary Artery Diseases

**A.**

- **% Cholesterol Efflux/4h**

**B.**

- **% Cholesterol Efflux/24h**

### Conclusions

- BioVision’s Cholesterol Efflux Assay Kit is a high-throughput screening assay for measuring cholesterol efflux in cells using fluorescently-labelled cholesterol.

- Cholesterol Efflux Assay provides a safe, sensitive and reproducible method of measuring cholesterol efflux.

- Several factors that modifies Reverse Cholesterol Transport and cholesterol efflux can be tested using this kit.

- BioVision’s Cholesterol Efflux Assay will address the importance of Reverse Cholesterol Transport and cholesterol efflux in atherosclerosis.