OrgFrontier™ Viable/Non-Viable Cells Separation Kit
(Catalog # K850-10, 10 preparations; Store at 4°C)

I. Introduction:
BioVision’s OrgFrontier™ viable/non-viable cell separation kit is designed to separate the viable (live) cells from non-viable (dead) cells in cultured cell preparations. This kit can be used to remove dead or damaged cells that can result from scraping, chemical treatment, or other procedures thereby damaging the healthy cells. The protocol can be performed and completed in an hour.

II. Applications:
- Separation of live and dead cells from active cultures
- Removal of dead cells from transfected cells
- Removal of dead cells from recently thawed cultures
- Removal of dead and damaged cells from chemically treated cultures
- Removal of cell clumps from cultured cells
- Separation of live cells from cells damaged by scraping

III. Sample Type:
- Culture cells: adherent or suspension cell lines

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>K850-10</th>
<th>Cap Code</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separation Buffer</td>
<td>2 x 70 ml</td>
<td>NM</td>
<td>K850-10-1</td>
</tr>
<tr>
<td>OptiPrep™ Density Gradient Medium</td>
<td>40 ml</td>
<td>NM</td>
<td>K850-10-2</td>
</tr>
</tbody>
</table>

V. User Supplied Reagents and Equipment:
- Table top or other cell culture compatible centrifuge. Refrigeration is not necessary.
- 15 ml Conical Centrifuge Tubes (Clear PET) tubes will give better visibility but are not required.

VI. Storage Conditions and Reagent Preparation:
Note: The sterility of the kit components is critical only if the processed cells are going to be re-cultured. Maintain sterile conditions by performing all the steps in a tissue culture/laminar flow hood using good aseptic techniques. Store the kit at 4°C. Protect from light. Read the entire protocol before performing the assay.
- Separation Buffer: Thaw completely prior to use. Store at -20°C or 4°C.
- OptiPrep™ Density Gradient Medium: Thaw completely and store at 4°C. Mix thoroughly before use.
- Gradient Working Solution (GWS): GWS is a 40% OptiPrep™ Solution. Prepare 6 ml GWS (equivalent to one isolation/preparation) by mixing OptiPrep™ with Separation Buffer at a 2:1 ratio (4 ml of OptiPrep™ and 2 ml Separation Buffer).
- 22.5% Gradient Solution: Prepare 3.2 ml of 22.5% Gradient Solution (equivalent to one preparation) by mixing GWS with Separation Buffer at 1:0.78 ratio (one-part GWS to 0.78 parts Separation Buffer). For one 15 ml tube, use 1.8 ml GWS and 1.4 ml Separation Buffer.

VII. Cell Separation Protocol:
1. Sample Preparation:
   a. Adherent cells (removed by Enzymatic/Non-enzymatic techniques)
      - Collect cells in a clean, sterile 15 ml conical tube. Wash flask with a 3 ml of Separation Buffer to remove any remaining cells and add to the conical tube.
      - Pellet cells by centrifugation (400 x g, for 5 min at RT).
      - Re-suspend the cell pellet in Separation Buffer so that the final volume is 1.5 ml.
   b. Adherent cells (removed by scraping)
      - Aspirate the growth medium and wash cells once with 4 ml Separation Buffer.
      - Add 5 ml of Separation Buffer (for T-75 flask). Using a cell scraper, carefully but thoroughly scrape to remove the cells.
      - Collect cells in a clean, sterile 15 ml conical tube.
      - Wash flask with a 3 ml of Separation Buffer to remove any remaining cells and add to the conical tube.
      - Pellet cells by centrifugation (400 x g, for 5 min at RT).
      - Re-suspend the cell pellet in Separation Buffer so that the final volume is 1.5 ml.
   c. Suspension Cells, Thawed Cells, and Treated, Unattached Cells
      - Collect cells by centrifugation (400 x g, for 5 min at RT).
      - Re-suspend the cell pellet in Separation Buffer in a 15 ml conical tube so that the final volume is 1.5 ml.  
      Note: For >10^6 cells, consider using a 50 ml centrifuge tube. Adjust volumes accordingly.
2. Viable/non-viable Cell Separation:
   a. Mix the 1.5 ml of the cell mixture (See Step 1) with 3.3 ml GWS. Mix gently until the two solutions are thoroughly blended.
b. Carefully layer 3 ml of the 22.5% Gradient Solution on top of the cell mixture. Do not mix or perturb the layers.

c. Carefully layer 1 ml of the Separation Buffer or Cell Culture media on top of the 22.5% solution. Do not mix or perturb the layers.

**Note:** When layering one solution on top of another, minimize the distance the new solution travels by placing your pipet as close to the previous layer as possible without touching it. Deliver the liquid slowly and consistently.

d. Centrifuge the tube at 800 x g for 20 min at RT.

e. Turn off the braking mechanism if possible. Or, set it to ‘low’.

f. At the end of the centrifugation, the healthy cell fraction will be in the visible band located at the interface of the Separation Buffer/22.5% gradient solutions.

g. Carefully remove most of the clear portion of the separation buffer gradient layer and then collect the band below it in a separate tube. These are healthy cells. **Note:** If clumps are visible and floating in the 22.5% solution, be careful to avoid them while collecting the band.

h. Dead cells will remain in the bottom layer. If user is interested in the dead cells, remove the entire upper layer. Wash pellet twice with 1X PBS (400 x g, for 5 min at RT). Resuspend the pellet in 1.5 ml Separation Buffer.

3. **Live Cell Clean Up:**

i. Add 4-5 vol. of cell culture media to the collected cells.

j. Centrifuge at 400 x g for 5 min at RT.

k. Resuspend pellet in media or buffer of choice. Cells are now ready to be used or re-cultured.

**Figures:** (a) Representation of a typical result using OptiPrep™ Density Gradient after centrifugation. Following centrifugation, isolated, healthy cells are localized at the interface between the Separation Buffer and 22.5% density layers (denoted by the bold blue shaded line).

(b) Viability percentages of adherent and suspension cell lines before and after separating non-viable cells using OptiPrep™ Density Gradient Medium and assayed using Live/Dead Cell Viability Assay Kit (Cat# K502). (c) Recovery percentages of viable cultured cells after separating non-viable cells using OptiPrep™ Density Gradient Medium.

**VIII. RELATED PRODUCTS**

Live/Dead Cell Viability Assay Kit (for Mammalian Cells) (K502)
Live/Dead Cell Staining Kit (K501)
EZViable™ Calcein AM Cell Viability Assay Kit (fluorometric) (K305)
VisionBlue™ Quick Cell Viability Fluorometric Assay Kit (K303)

---

FOR RESEARCH USE ONLY! Not to be used on humans.