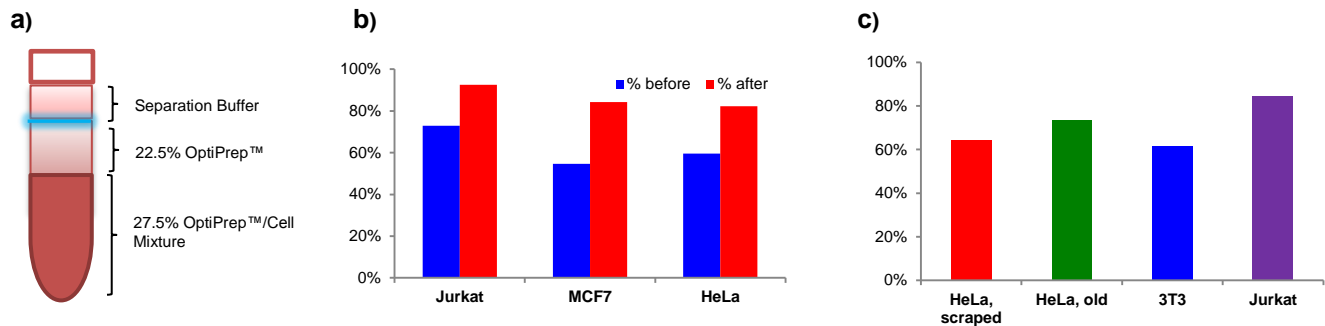




- b. Carefully layer 3 ml of the 22.5% Gradient Solution on top of the cell mixture. Do not mix or perturb the layers.
- c. Carefully layer 1 ml of the Separation Buffer or Cell Culture media on top of the 22.5% solution. Do not mix or perturb the layers.  
**Note:** When layering one solution on top 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.**