3D Biotek
Add Extra Dimension to Your Innovation

675 US Highway One
North Brunswick, NJ 08902
Tel: 732-729-6270
Fax: 732-745-7270
http://www.3DBiotek.com

Cell Detachment Protocol

Any detachment method that you are currently using with 2D cultured cells can be used to detach cells cultured on our 3D Insert™ scaffolds!

Cells cultured on 3D Biotek's PCL and PS scaffolds secrete significant extracellular matrix (ECM), it may be necessary to modify your protocol accordingly. We have listed some suggestions to increase your cell yield.

1. Remove cell culture media.
2. Rinse scaffolds 2X's with 1XPBS.
3. Initially, we recommend that you try the following enzymes. It may be necessary to alter enzyme concentrations.
   - Trypsin (+/- EDTA) solution
   - Collagenase solution
   - Mixture of trypsin (+/- EDTA) and collagenase solutions
4. Completely immerse the scaffold in your enzyme solution.
5. Place the cell culture plate with scaffolds into incubator at 37ºC for 10-30 minutes. **Please note that agitating your plate with on a shaker or nutator, etc., during this time can enhance cell detachment!**
6. Monitor cell detachment.
7. After cells have detached, add growth media at a volume equal to the original enzyme solution.
8. Gently pipette the cell suspension up and down ~5X's within the scaffold in order to flush out the remaining cells from the scaffold.
9. Spin down cells for 3 min at 1,000 rpm and resuspend in growth media.
10. Cells are ready to be used for reseeding or experimental analysis.

It has been found that all of these enzymes are effective in detaching cells from our 3D scaffolds. Using Trypan Blue Exclusion Assay, we have found that cells cultured in 3D scaffolds are very resistant to enzymatic damage. The number of dead cells (positive staining) in 2D compared with 3D is significantly higher after Trypsin/EDTA treatment (Fig 1). After 30 min of trypsin treatment, there is no cell yield from 2D culture (Fig 1).

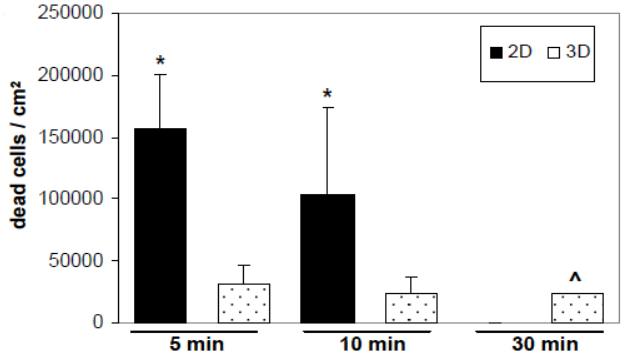

Figure 1. Trypsin/EDTA treatment time-course. After 4 days of culture, NIH-3T3 cells were lifted with Trypsin/EDTA solution and dead cells were counted using the Trypan Blue Exclusion Assay. *, $p \leq 0.05$, significantly more dead cells were found in 2D compared with 3D scaffolds; ^, $p \leq 0.05$,