Detachment of cells cultured on Fibrous Collagen Surfaces

General note about detachment enzymes: Cells can be detached from the collagen surface by using different detachment enzymes. Do not re-use the collagen membranes or tubes after enzymatic treatment.

Herein a summary about the properties (advantages / disadvantages) of the most commonly used cell detachment enzymes can be found. To detach cells grown on the Collagen Cell Carrier (CCC) or on the Collagen BioTubes (CBT), Trypsin with and without EDTA (in the following called Trypsin), TrypLE Select® or Accutase® can be used.

Trypsin: Is produced from porcine pancreas and is a mixture of proteolytic enzymes, mainly Trypsin will be found but also Elastase and Chymotrypsin. Thus it shows variations in detachment performance from lot to lot. Too long treatment of cells with Trypsin will lead to irreversible cell damage. Most of the surface proteins will be destroyed by Trypsin during the detachment procedure. Trypsin needs to be actively inactivated to stop the reaction. Inactivation can be performed by using serum, serum containing medium or soybean Trypsin inhibitor (often called TNS – Trypsin neutralization solution).

Accutase®: Is a mixture of enzymes with proteolytic and collagenolytic activity isolated from crabs and is free of contaminating mammalian components. Accutase® can be used as a direct Trypsin replacement. It is a very gentle detachment enzyme (no obvious differences in cell viability can be found after treatment of cells comparing 15 to 50 min incubation time at 37°C). Accutase® allows cell detachment while most of the surface proteins stay intact. Since it will be inactivated automatically after one hour at 37°C it has to be stored at 4°C and repeated thawing / freezing cycles should be avoided. No actively inactivation of Accutase® is necessary to stop the reaction.

TrypLE Select®: Is a recombinantly produced Trypsin and can be used as a direct Trypsin replacement. Due to its non-animal or human origin, it is free of contaminating viruses or prion proteins. It can be stored at room temperature and will be stable for up to 6 months. Compared to Trypsin it is not that harsh and the cell viability after passaging will be good. To finish the detachment reaction only a dilution of TrypLE with buffer or medium is necessary.

Please note: The detachment protocol has to be optimized for each cell type.

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Revision Date: 1/18/2017

1. Aspirate the culture medium from the well thereby always avoid damaging the collagen surface.
2. Wash the cells by adding 100 µL / cm^2 PBS (w/o Ca^{2+} and Mg^{2+}) pre-warmed at least to room temperature. To rinse the cells, carefully rotate the cell culture vessel and aspirate the supernatant. Never pipette directly on top of the cells, add liquids gently along the sidewall.
3. Add 50 µL / cm^2 of preferred detachment enzyme.
4. Incubate at 37°C until the cells are rounded and start to detach (rounded cells are visible by light microscopy). Cell detachment has to be monitored under the microscope!
5. Tap the cell culture vessel to accelerate cell detachment.
6. Detachment reaction needs to be stopped depending on detachment enzyme used.

Trypsin:	add the same volume Trypsin inhibitor (see general notes).
Accutase®:	add one to two volumes of medium to the cells (if you are in the need to save medium also PBS (w/o Ca^{2+} and Mg^{2+}) can be used).
TrypLE Select®:	add one to two volumes of medium to the cells (if you are in the need to save medium also PBS (w/o Ca^{2+} and Mg^{2+}) can be used).

1. To remove all cells and to get a single cell suspension rinse the collagensurface two to three times with the suspension.
2. Transfer the cell suspension to a centrifugation tube.
3. Centrifuge the cells 5 min, 200 × g at room temperature.
4. Aspirate the supernatant and resuspend the cell pellet in an appropriate volume of pre-warmed medium.
5. If you want to reseed the cells count them and seed viable cells in the required seeding density.

Please note: The protocol for detachment of cells from CBT needs to be optimized by the user.

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