USER GUIDE

ENZYMATIC CELL DETACHMENT

Detachment of cells cultured on fibrous collagen surfaces

Cells can be detached from a collagen matrix by using different enzymes. We have summarized the properties (advantages & disadvantages) of the most commonly used cell detachment enzymes below.

To detach cells grown on the Collagen Cell Carrier® (CCC) or on Collagen BioTubes (CBT), trypsin with and without EDTA (in the following called trypsin), TrypLE Select® or Accutase ® can be used as described in the protocol below.

Please note:

- Collagen membranes or tubes can't be re-used after enzymatic treatment.
- All detachment protocols need to be optimized according to need for each cell type, and for the CCC or CBT respectively.

Cells can be removed enzymatically from collagen matrices such as the CCC.

GENERAL OVERVIEW ON DETACHMENT ENZYMES

Trypsin: Produced from porcine pancreas, products are a mixture of proteolytic enzymes, mainly trypsin but also elastase and chymotrypsin, which might lead to variations in detachment performance from lot to lot. Treatment of cells with trypsin for a longer time period 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, e.g. by serum, serum containing medium or soybean trypsin inhibitor (also 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 active inactivation of Accutase® is necessary to stop the reaction.

TrypLE Select®: Is a recombinant trypsin that can be used as direct trypsin replacement. Due to its non-animal or human origin, it is risk-free for 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 cell viability after passaging is good. To stop the detachment reaction a dilution of TrypLE with buffer or medium is sufficient.

Required material

- Detachment enzyme (trypsin, Accutase®, or TrypLE Select®)
- Detachment enzyme inhibitor (if applicable)
- Prewarmed PBS w/o Ca^{2+} and Mg^{2+}
- Cell culture medium

Disclaimer

All data and recommendations correspond to the present state of our knowledge; they are published without engagement. We reserve the right to make alterations and additions in line with technical developments without prior notice. The customer is obliged to check whether our products meet the technical requirements.

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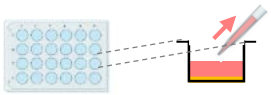
CELL DETACHMENT PROTOCOL

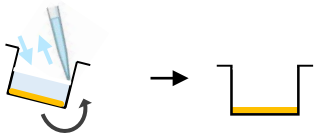
STEP 1

Aspirate the culture medium from the well and avoid damaging the collagen surface.



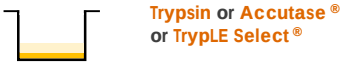
STEP 2

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.



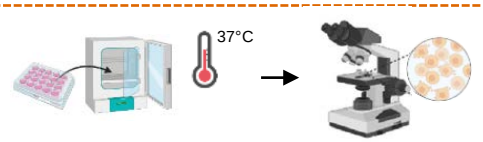
STEP 3

Add 50 µL / cm^2 of preferred detachment enzyme.



STEP 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!



STEP 5

Tap the cell culture vessel to accelerate cell detachment.

Detachment reaction needs to be stopped depending on the enzyme used:

>> STOP ENZYME REACTION <<

STEP 6

Trypsin:
Add the same volume trypsin inhibitor (see general notes).

Accutase®:
Add 1-2 volumes of medium to the cells. If you need to save medium also PBS w/o Ca^{2+} and Mg^{2+} can be used.

TrypLE Select®:
add 1-2 volumes of medium to the cells. If you need to save medium also PBS w/o Ca^{2+} and Mg^{2+} can be used.

STEP 7

To remove all cells and to get a single cell suspension rinse the collagen surface two to three times with the suspension.

STEP 8

Transfer the cell suspension to a centrifugation tube.

STEP 9

Centrifuge the cells 5 min., 200 × g at room temperature.

STEP 10

Aspirate the supernatant and resuspend the cell pellet in an appropriate volume of pre-warmed medium.

STEP 11

If you want to reseed the cells count them and seed viable cells in the required seeding density.

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