Technical Info

Well advised with Roth.

Use of trypsin in cell passage / subcultivation of cells.

Cells in culture need to be passaged (split, subcultured) routinely. In case of adherent cells, they are detached from culture surfaces and partly transferred to new culture vials containing fresh culture medium. This prevents the cells from being exposed to too tight cell-cell contacts and reducing their cell division rate due to the resulting cell contact inhibition. How often cells need to be passaged depends on the cell type. Usually, cells are transferred to new culture vials at standard culture conditions (37°C, 5% CO_2) at approximately 80% confluence (surface coverage). Cell dissociation or cell detachment from culture surfaces using trypsin or trypsin/EDTA solutions is by far the most common method in routine cell culture.

Trypsin is a digestive enzyme belonging to the serine proteases with a molecular weight of 23.3 kDa, which is derived from the pancreas of pigs. It develops its greatest activity at a pH optimum of 7.5-8.5 and a temperature of 37 °C. *In vivo*, trypsin is produced in the pancreas in the form of the inactive zymogen trypsinogen. After reaching the duodenum, activation of trypsinogen to trypsin is catalyzed by enteropeptidase. Once small amounts of trypsin are present, there is increased autoactivation of trypsinogen by trypsin, which in turn accelerates the formation of trypsin. The functional task of trypsin is the hydrolytic cleavage of proteins and peptides. In this process, trypsin cleaves at the C-terminal side of lysine and arginine residues. The exception here are the Lys-Pro and Arg-Pro sequences, which are not cut. Also, trypsin attacks peptide bonds between a basic (Lys, Arg) and an acidic amino acid (Glu, Asp) only slowly.

Trypsinization thus leads to the cleavage of adhesion proteins through which the cells adhere to the surface of culture vials. However, trypsin also has undesirable side effects, which is why it is important to stop trypsinization after a certain time. For this purpose, an inhibition step or neutralization step is needed. Trypsin inhibition is achieved either by FCS/FBS, if serum-containing media are used, or by trypsin inhibitors.

Trypsin is often used in routine cell culture in combination with **EDTA.** EDTA is a chelating agent and enhances the ability of trypsin to detach adherent cells. EDTA binds calcium and magnesium, which additionally weakens cell-cell contacts. This favors the hydrolysis of specific peptide bonds by trypsin.

Recommended reagents from Carl Roth

ROTI®Cell Media	
ROTI®Cell Trypsin Solution (1x)	Order No. 1Y17
ROTI®Cell Trypsin Solution (10x)	Order No. 1Y16
ROTI®Cell Trypsin/EDTA Solution (1x)	Order No. 1Y1A
ROTI®Cell Trypsin/EDTA Solution (10x)	Order No. 1Y19
Trypsin inhibitor	Order No. 2949
ROTI®Cell DPBS (without Ca/Mg)	Order No. 9124
ROTI®Cell PBS	Order No. 9143
ROTI®Cell Water	Order No. 9186

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Common protocol for cell passage:

Trypsinization must be performed under a laminar flow in sterile conditions.

Depending on the cell type, there may be variations in incubation times and trypsin concentrations. 10x solutions should be diluted to a 1x solution beforehand.

1. Thaw Trypsin or Trypsin/EDTA solution in a water bath at +37°C or overnight at +2°C to +8°C. Homogenize by gentle swirling.
2. Allow PBS / DPBS without Ca^{2+} and Mg^{2+} and appropriate cell culture medium (see recommended reagents) to warm up at room temperature if previously stored at +4°C.
3. Under the light microscope, verify that the cells have reached the desired confluence and are in a vital state without contamination.
4. Carefully pipette off the medium in the cell culture flask, leaving the cell layer as untouched as possible. **Tip:** Tilt the cell culture flask and remove the medium from only one corner of the vial.
5. Wash cell layer with PBS / DPBS without Ca^{2+} and Mg^{2+} (approx. 5 ml salt solution per 25 cm^2). **Tip:** Allow the saline solution to flow into the vial along the cell free side of the cell culture flask. Swirl the cell culture flask slightly so that the saline solution gently washes the cell layer. Then carefully remove the saline solution (see tip in 4.).
6. Add warmed trypsin or trypsin/EDTA solution to the cell culture flask so that the cell layer is completely covered (200 µl is sufficient for 25 cm^2 cell layer).
7. Incubate the trypsin or trypsin/EDTA solution on the cell layer for approx. 3 min at 37°C in the incubator. For a gentler treatment, incubate at room temperature with extended incubation time. As soon as the cells appear rounded and float on the bottom of the flask, trypsinization can be stopped. Check under the light microscope whether all cells are detached. **Tip:** By gently tapping the cell culture flask, the cells detach much faster, so that the trypsinization can be stopped earlier.
8. Inhibit trypsin by directly taking up the cells in fresh cell culture medium containing serum (approx. 5 ml for 25 cm^2 cell layer). Unless working with serum (FCS/FBS), trypsin inhibition is performed using a trypsin inhibitor solution (1 mg/ml in water or PBS) in a 1:1 ratio with the trypsin solution and subsequent uptake in cell culture medium. **Tip:** Rinse the fresh medium from the pipette with a little more pressure directly over the previously cell coated bottom of the bottle, so that even the last cells are detached from the vial. By reabsorbing the resulting cell suspension, this rinsing step can be repeated (approx. 3 x) and thus the entire bottom of the bottle can be "rinsed off". In this step also possible cell clumps are dissolved.
9. Transfer the cell suspension into a fresh falcon and centrifuge cells (5 min, 1000 rpm). **Tip:** During the centrifugation step, provide new cell culture flasks or even well plates, label them and supply medium if necessary.
10. After centrifugation, remove excess and resuspend cell pellet in fresh cell culture medium. Use the appropriate amount of medium to achieve the desired cell density. **Tip**: For cell experiments, the cell number is determined at this point using a Neubauer counting chamber (add 10 µl cell suspension to counting chamber).
11. Cell suspension can then be transferred to new cell culture flasks or well plates. To achieve the appropriate cell density, medium can be provided for this purpose (see tip in step 9). With the help of a slight north, south, east, west movement of the cell culture flask on the laminar flow table, the cells are evenly distributed. Caution: The cell suspension should not get into the neck of the bottle.

Note: The time between the single steps should be as short as possible so that the cells do not remain dry for too long.

L.H., 16.11.22