Technical Info

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Cell Passage and Use of Trypsin

In cell culture, **passaging** is the process of sub-culturing cells in order to produce large number of cells from pre-existing ones. Passaging (also known as subculture or splitting cells) involves splitting the cells and transferring a small number into each new vessel. Cells can be cultured for a longer time if they are split regularly, as it avoids the senescence associated with prolonged high cell density.

Suspension cultures are easily passaged with a small amount of culture containing a few cells diluted in a larger volume of fresh media. For adherent cultures, cells first need to be detached, commonly done with a mixture of trypsin-EDTA. A small number of detached cells can then be used to seed a new culture, while the rest is discarded. Also, the amount of cultured cells can easily be enlarged by distributing all cells to fresh flasks.

In the average lab, **adherent cells** are grown in petri dishes, multi-wells plates or culture flasks, with FCS containing culture media at 37 °C with 5 % CO_2. In the case of more or less fast growing cells like HeLa, a freshly seeded plate will reach confluency in two or three days. If nothing is done, the food will run out and the cells will die shortly thereafter, so passaging is required. Thus, the media is removed, the cells are washed with phosphate buffered saline, then trypsin is added to make the cells detach from the bottom of the plate. Trypsin works best in a warm surrounding, so the plate is incubated for five minutes in 37 °C. In order to stop the reaction, trypsin inhibitor stock solution is added. Then, cells are resuspended in PSB and cells are counted. An appropriate number of cells in suspension is then transferred new plates,fresh media is added to each plate, and the new plates are incubated for the next growth phase.

EDTA, a chelator of divalent cations, is used combined with trypsin prior to passaging in order to solubilize adherent cells from the culture vessel surface into suspension. EDTA binds calcium and magnesium which aid in cell-cell adhesion, additionally allowing trypsin to more efficiently hydrolyze specific peptide bonds. A very gentle cell dissociation may be performed using EDTA directly without addition of trypsin.

For best results, cells are kept less than 100 % (log phase of growth) but more than 10 % confluent. Cells die if they get too lonely (“apoptotic death of neglect”) or much too crowded (death by metabolic decay).

Trypsin is an endopeptidase produced by the gastro-intestines of mammals, and has an optimal operating pH of about 8 and an optimal operating temperature of about 37 °C. *In vivo,* trypsin is produced in the pancreas in the form of inactive zymogen, trypsinogen. After secretion into the duodenum, the enzyme enteropeptidase activates a small number of the enzymes into trypsin by proteolytic cleavage, followed by autocatalysis of trypsins to activate the whole secreted mass. Then, trypsin acts to hydrolyse pepsin-digested peptides by hydrolysis of peptide bonds.
The aspartate residue (Asp 189) located in the catalytic pocket (S1) of trypsins is responsible for attracting and stabilizing positively-charged lysine and/or arginine. Thus, trypsin predominantly cleaves proteins C-terminally of the amino acids lysine and arginine, except when either is followed by proline.

Trypsins should be stored at very cold temperatures (between −20 °C and −80 °C) or at a pH of 3 in order to prevent autolysis.

Carl Roth GmbH + Co. KG
Labware · Life Science · Chemicals
Schoemperlenstraße 3–5 · D-76185 Karlsruhe
Phone +49 721 5606-0 · Fax +49 721 5606-149
P.O.Box 100121
76231 Karlsruhe
info@carlroth.com
www.carlroth.com

Protocol Cell Passage

Only use endotoxin-free solutions and reagents for all procedures mentioned here (Roth CELLPURE® quality)

1. Remove and discard the wasted medium.
2. Wash cells in 1x PBS or 1xDPBS
3. Apply trypsin/EDTA* solution, ca. 1 ml / 25 cm^2 growth area.
4. Incubate at 37 °C for few minutes, until cells start to lift from the plastic surface of the slanted flask
5. Add 1 ml (per ml trypsin) trypsin inhibitor solution (1 mg/ml in water or PBS)
6. Add 8 ml (per ml trypsin) PBS, resuspend cells
7. *Optional:* Count cells in a Neubauer chamber
8. Leave the intended amount of cells (usually ¼ to 1/3 volume) in the flask or transfer to a fresh flask. Discard or freeze the residual cell solution.
9. Add fresh medium to the cells, cultivate further in the incubator.

* For very gentle cell dissociation, pure EDTA in PBS or DPBS may be used.
Incubation time increases significantly, while addition of a trypsin inhibitor solution is not necessary.

Preparation of a 10 % Trypsin Stock Solution

Solubilise 10 g trypsin in 100 ml 10 x trypsin buffer
Sterilize by filtration (0.2 µm) and store in aliquots (e.g. 10 ml each) at -20 °C or -80 °C.
Durability approx. 1 year.

10 x Trypsin buffer

80 g NaCl
3 g KCl
0,73 g Na_2HPO_3 x 2 H_2O
0.2 g KH_2PO_3
20 g Glucose
7 g EDTA

Fill with water ad 1 Litre, pH 7.2 (NaOH, Art. No. K021)

Preparation of working solution

Dilute stock solution 1:10 with sterile distilled water. Store at +4 °C.

Durability approx. 1 week.

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Recommended Reagents

ROTI®Cell DPBS/EDTA	Ord. No. 9135
ROTI®Cell PBS/EDTA	Ord. No. 9152
ROTI®Cell Wasser	Ord. No. 9186
ROTI®Cell PBS	Ord. No. 9143
ROTI®Cell 10x PBS	Ord. No. 9150
ROTI®Cell DPBS without Ca/Mg	Ord. No. 9124
Trypsin	Ord. No. 2193
Trypsin inhibitor	Ord. No. 2949
NaCl (CELLPURE®)	Ord. No. HN00
KCl (CELLPURE®)	Ord. No. HN02
Glucose (CELLPURE®)	Ord. No. HN06
Disodium hydrogenphosphate dihydrat	Ord. No. 4984
Potassium dihydrogenphosphate	Ord. No. 3904
EDTA	Ord. No. 8043

We recommend using endotoxin-free water for preparation of stock- und working solution. Water made by common distillers frequently is polluted with bacteria. Although these microorganisms are removed by sterile filtration, the released endotoxins may affect cultured cells heavily. Endotoxin-free water can be obtained under order numbers 3255 and 9186 (CELLPURE®).

gh 02/2020

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