

Trypsin/EDTA (10x solution)

#GTC06.0100
(for research only)

Product:	Filter sterilized solution of 0.5% Trypsin from porcine pancreas in 10x Dulbecco's PBS (pH 7.0-7.6) with EDTA, without Ca^{2+} , without Mg^{2+} , and without phenol red. Concentrated cell detachment solution for breaking down cell adhesion structures.
Quantity:	100ml
Appearance:	Clear colourless solution.
Storage:	-20°C for up to 2 years.
Specifications:	Activity: > 5000 BAEE U/ml pH: 7.0–7.6 Osmolality: 280-360 mOsm/kg Sterility: sterile Parvovirus test : negative Performance: Cell Detachment

Preparation of 1x solution from 10x concentrated solution

Note: The entire protocol should be carried out in a laminar flow hood, using proper aseptic techniques.

1. Pre-warm the 10x concentrated Trypsin/EDTA solution to 37°C by placing in a water bath.
2. Once thawed, aseptically dilute 100ml of the 10x concentrated solution with 850ml of a sterile Ca^{2+} - and Mg^{2+} -free salt solution (e.g. Dulbecco's PBS) and mix well.
3. If necessary, adjust pH to 7.2-7.8 with 1M HCl or 1M NaOH.
4. Adjust the volume to 1000ml with sterile Ca^{2+} - and Mg^{2+} -free salt solution (e.g. Dulbecco's PBS) and mix well.
5. Dispense the solution into sterile container and store at -20°C.

Typical trypsinization protocol

(depends on cell line and should be adapted conform)

Prior to start, pre-warm all solutions (Trypsin/EDTA (1x) and PBS (without Ca^{2+} and without Mg^{2+})) to 37°C by placing in a water bath. The entire protocol should be carried out in a laminar flow hood, using proper aseptic techniques.

1. Carefully aspirate off all the culture media from the flask, without disturbing the cell layer or letting the cell layer dry. Proceed immediately with washing the cells with PBS (rinse with 5 ml for a T25 flask and with 10 ml for a T75 flask).
2. Immediately after, add sufficient Trypsin/EDTA (1x) solution to cover the cells. For a T25 flask, 0,5ml* should be enough. Incubate the flask at 37°C for 2-3 minutes in the cell culture incubator. Check cell morphology visually (microscope) to verify if cell have rounded, if not continue incubation. The solution in the flask will appear cloudy. Incubation time should be kept at a minimum and overexposure should be avoided, as Trypsin can cause cellular damage. The required time depends among others on cell type, culture age, cell density, and the serum concentration in the growth medium).
3. Once cells are rounded and detached, beat the flask against the palm of your hand to loosen any remaining attached cells
4. Wash out all the cells from the surface by pipetting the fresh complete cell culture medium (5ml) all over the surface. Gently disperse the cells to break cell clumps. Take a sample to determine the viable cell density, and add aliquots of detached cells to fresh culture media in new flasks.
*) When using more, e.g. 2-4ml, then in step 4, you must add more serum containing medium to inhibit trypsin after digestion has been completed, or neutralize excess of trypsin by adding trypsin inhibitor.

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