Trypsin/EDTA (10X Solution)

#GTC06.0100 (100ml)
(FOR RESEARCH ONLY)

Product: Filter sterilized solution of 0.5% Trypsin form porcine pancreas in 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: #GTC06.0100 comprises 100ml of 10X Trypsin/EDTA in DPBS

Applications: Cell detachment (trypsinization) of cells that are attached to plasticware.

Appearance: Clear colourless solution.

Specifications:

Activity:	>5000 BAEE U/ml
pH:	7.0-7.6
Osmolality:	280-360m Osm/kg
Sterility:	sterile
Parvovirus:	negative

Storage: -20ºC for up to 2 years.

Prior to use:

Pre-warm all solutions (Trypsin/EDTA (10x) and PBS (without Ca^{2+} and without Mg^{2+})) to 37ºC by placing in a water bath. The entire protocol should be carried out in a laminar flow hood, using proper aseptic techniques. Once thawed, dilute 100ml of the 10X concentrated Trypsin/EDTA solution with 850ml of a sterile Ca2+- and Mg2+-free salt solution (e.g. DPBS) and mix well. If necessary, adjust pH to 7.2-7.8 with 1M HCl or 1M NaOH. Adjust volume with DPBS or equivalent to 1L and mix well. Dispense work solution into a sterile container and store at -20ºC or continue with trypsinization.

Typical Trypsinization Protocol

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.*