

Trypsin-EDTA

Product Information

CytoSpring

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Background Trypsin is a member of the serine protease family. It cleaves peptides on the C-terminal side of Lysine or Arginine. Trypsin is by far the most commonly used enzyme in tissue culture to release the adherent cells from culture vessel surface and/or to disaggregate tissue into single cell suspension. It is tolerated well by many cell types and its enzymatic activity can be neutralized easily by serum in culture medium.

Cell-cell and cell-matrix interaction depends on the adhesion molecules, which in turn, relies on the presence of calcium. Tissue culture media contains calcium and magnesium ions, fetal bovine serum contains proteins that are trypsin inhibitors. Both Mg^{2+} and Ca^{2+} inhibit trypsin. To weaken the function of adhesion molecules, EDTA is frequently included in the trypsin solution for its function as divalent cations chelator. To reduce the concentration of divalent cations and proteins that inhibit trypsin action, before splitting cells with trypsin, the cells should be washed with PBS without Ca^{2+}/Mg^{2+} .

The pH optimum of trypsin function is from 7.6 to 8.5, with the maximal activity falling within the narrow pH range of 8 to 8.2¹. Phenol red is often included in the trypsin-EDTA solution, giving a pinkish color under this pH range. To preserve enzymatic activity, trypsin-EDTA is normally shipped on dry ice. Occasionally, significant amount of CO_2 built up in the package can enter into the trypsin container and thus lower the pH, giving an orange color indicating acidic (pH nears 6.5), which renders trypsin less effective. To enhance the activity, simply adjusting the pH to 7.4 - 7.6 with NaOH and filter-sterilize if sterility is required.

Storage Product is shipped on dry ice and storage at $-20^{\circ}C$ is recommended. Avoid leaving at room temperature for extended time as trypsin can self-digest¹.

Product The table below lists the products of trypsin-EDTA that CytoSpring carries. Trypsin 1x solutions can range from 0.025% to 0.5% depending on application. Our trypsin is derived from porcine pancreases. All products are sterile-filtered and cell culture tested.

Product #	T0111	T0113	T0131	T0133
Description	Trypsin-EDTA, 1X	Trypsin-EDTA, 1X	Trypsin-EDTA, 1X	Trypsin-EDTA, 1X
Conc. of trypsin	0.05g (0.05%)	0.05g (0.05%)	0.25g (0.25%)	0.25g (0.25%)
Conc. of EDTA	0.53mM	0.53mM	1mM	1mM
volume	100ml	500ml	100ml	500ml
Buffer type	HBSS* w/o Ca^{2+}/Mg^{2+}	HBSS* w/o Ca^{2+}/Mg^{2+}	HBSS* w/o Ca^{2+}/Mg^{2+}	HBSS* w/o Ca^{2+}/Mg^{2+}

*HBSS: Hank's balanced salt solution².

Reference

1. Buck FF, Vithayathil AJ, Bier M, Nord FF. On the mechanism of enzyme action. 73. Studies on trypsins from beef, sheep and pig pancreas. Arch Biochem Biophys. 1962, 97:417-424.
2. Hanks J. Hanks' balance salt solution and pH control. 1976, 3:3.

Q & A

What concentration of trypsin-EDTA solution should I use when passage a cell line that I am not familiar with?

A. Because trypsin cuts proteins, it is important not to allow cells in contact with trypsin longer than necessary, as cell membrane can be damaged, resulting in reduced cell viability. We recommend customers to start with low concentration and incubate for a short period of time (~ 5 min) if customers are unfamiliar with the cell line under investigation. The trypsin products that CytoSpring carries are derived from porcine. Thus, batch-to-batch variation can occur. Different cell lines may need trypsin at different potency to be lifted efficiently from culture vessel surface. Trypsin at different concentrations (mixed with a fixed amount of EDTA) is available commercially.

Can I wash cells with trypsin-EDTA solution instead of DPBS?

A. Rinsing cells briefly with trypsin solution can achieve the same goal as with DPBS, However, trypsin is relatively more expensive. DPBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$ is an economic choice.