

Product Information

Trypsin-EDTA Solution 10X

0.5% trypsin, 0.2% EDTA, trypsin gamma irradiated by SER-TAIN™ Process, without phenol red, in saline
CATALOG NO. 59418C

Description

Trypsin is a porcine pancreas-derived enzyme that is commonly used for the dissociation and disaggregation of anchorage-dependent mammalian cells and tissues. The concentration of trypsin necessary to dislodge cells from their substrate is dependent primarily on the cell type and the age of the culture.

Ethylenediaminetetracetic acid (EDTA), a chelating agent, often is added to trypsin solutions to enhance enzymatic activity by neutralizing calcium and magnesium ions that enhance cell-to-cell adhesion and obscure the peptide bonds on which trypsin acts.

This 10X concentrated solution can be diluted with calcium- and magnesium-free Dulbecco's Phosphate Buffered Saline (DPBS).

SAFC Biosciences® has validated a process using gamma radiation to significantly reduce the risks associated with adventitious agents such as Porcine Parvovirus (PPV), Porcine Respiratory and Reproductive Syndrome (PRRS) and *Mycoplasma hyorhinis* while maintaining product performance. The use of gamma irradiated trypsin requires no change to the end user's methods or procedures while giving additional assurance against microbial contaminants associated with animal-derived products.

All trypsin is obtained from the United States or other countries deemed free of Bovine Spongiform Encephalopathy (BSE).

Precautions

Use aseptic technique when handling or supplementing this solution. This product is for further manufacturing use. THIS PRODUCT IS NOT INTENDED FOR HUMAN OR THERAPEUTIC USE.

Storage

Store trypsin at -10 to -40 C. Do not use after expiration date. Repeated cycles of freezing and thawing reduce enzymatic activity and should be avoided.

Formulation

Component (all components measured in mg/L)	
INORGANIC SALTS	
EDTA 4Na monohydrate	2000.000
Sodium chloride	8500.000
OTHER	
Trypsin porcine (1:250)	5000.000

Indications of Deterioration

Trypsin solutions should be clear of particulates and flocculent material. Do not use if solution is cloudy or contains precipitate. Other evidence of deterioration may include color change or degradation of physical or performance characteristics.

Preparation Instructions

How to dilute trypsin 10X solutions:

1. Frozen products can be thawed either in a 37 C water bath or overnight at 2 to 8 C.
2. Aseptically transfer 100 mL of trypsin 10X to a sterile 1 L container.
3. Add 800 mL of a sterile calcium- and magnesium-free salt solution (as listed below) to the container.
 - Dulbecco's Phosphate Buffered Saline (DPBS) (Catalog No. 59321C), or
 - Hanks' Balanced Salt Solution (HBSS) (Catalog No. 55025C).
4. Mix well for several minutes. Determine the pH of a small sample. If necessary, adjust the pH to 7.2 - 7.8 with NaOH 1N (Catalog No. 59223C) or HCl 1N .

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- Bring the final volume up to 1000 mL with the salt solution chosen from Step 3 and either sterile filter into appropriate use volumes or, after sterile filtration, aseptically dispense into smaller volumes.
- Store trypsin at -10 to -40 C.

Methods for Use

- Frozen trypsin can be thawed either in a 37 C water bath or overnight at 2 to 8 C.
- Aspirate and discard the spent medium from the culture vessel.
- Rinse the monolayer with either a small amount of trypsin or a calcium- and magnesium-free balanced salt solution; aspirate and discard.
- Add enough trypsin solution, prewarmed in a 37 C water bath, to completely cover the cell monolayer.
- Incubate the flask at 37 C, or for more sensitive cultures, at room temperature or 2 to 8 C.
- When the trypsinization process is complete, cells will appear rounded upon microscopic examination and the solution in the flask will appear cloudy. Check the flask often to avoid overexposure which can damage the cells.
- The trypsin should be neutralized either with serum-containing medium or trypsin inhibitor. Gently centrifuge the cell suspension and discard the trypsin-containing supernatant.
- Resuspend the cell pellet with fresh medium and count or culture as desired.

Characteristics

Adventitious Viral Agents (AVA) (PPV)

None detected in bulk powder raw material

Appearance

Clear colorless solution

Endotoxin (at 1X concentration)

≤ 100.0 EU/mL

Enzymatic Activity

Cells dislodged ≤ 30 minutes

Mycoplasma

None detected

Osmolality (at 1X concentration)

270 - 325 mOsm/kg H₂O

pH (at 25 C)

7.2 - 7.8

Sterility

No microbial growth detected

References

- Hodges, G. M., Linvingston, D. C., and Franks, L. M., *J. Cell Sci.* (1973) 12:887.
- McKeehan, W. L., *Cell Biol. Intl. Rep.* (1977) 1:335.
- Safton, B. M. and Rubin, H., *Nature* (1970) 227:843.

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