Technical Data Sheet

Trypsin 1:250 from porcine pancreas

for biochemistry

Order number: **1004**

Trypsin 1:250 is a proteolytic enzyme (digestive enzyme) from the porcine pancreas. It is a serine protease that cuts proteins and peptide chains mainly at the carboxyl side of the amino acids lysine or arginine. The optimum pH of trypsin is slightly above 8 and the ideal working temperature is 37 °C.

Applications

1. **Cell Detachment.**

In cell biology, trypsin solutions are used widely for dissociation of tissues and cell monolayers.
For passaging/subculturing, adherent growing cells must be detached (from the cell culture dishes and flasks) and the individual cells must be separated from each other. This is done by trypsin: the protease hydrolyses the proteins that mediate binding to the surfaces. The so-called trypsinization is a routine application in the propagation of adherent cells and is used when the cultures have to be transferred to a new vessel.
The concentration of trypsin required to detach cells from a surface depends on the cell type and the extent of cell aging.
Typical formulations are:

- Trypsin 0.25% for continuous and strongly adherent cell lines
- Trypsin 0.25% + 0.05 % (~ 1 mM) EDTA for strongly adherent early-passage cell lines, distinct tumor cell lines and for thick cultures with multiple cell layers
- Trypsin 0.05% + 0.53 mM (0.02%) EDTA for continuous cell lines when cell surface protein integrity is important

In general, the less trypsin, the more gentle the procedure is for the cells. EDTA as an additive captures calcium and magnesium ions on the cell surface and is an important component when it comes to breaking cell-cell adhesions. Furthermore, EDTA addition increases the proteolytic activity of trypsin.
Long-term incubation with high trypsin concentration and/or for a long period of time leads to cell membrane damages and finally to cell death. Following the detachment process, trypsin must therefore be inactivated by a suitable trypsin inhibitor or by adding serum (contains protease inhibitors and divalent cations).

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2. **In-gel digestion**

Trypsin is used in proteomics to generate peptides for mass spectrometric analysis.

3. **GTG banding**

Trypsin is used in karyotyping to improve the visibility of chromosomes by applying the Giemsa-Trypsin-Giemsa banding technique.

Storage and reconstitution

Store trypsin powder at 2 to 8 °C. Make sure there is no contact with moisture. Trypsin powder remains stable for years.

For reconstitution of trypsin powder, a balanced salt solution, e.g. PBS or HBSS without Ca^{2+} or Mg^{2+} ions should be used. Solutions of trypsin must be frozen at -20°C after preparation to prevent autolysis. Avoid repeated freezing and thawing. Frozen in sterile aliquots, the solutions can be used for up to one year.

Trypsin activity

There are different methods to determine trypsin activity. Most of them are based on a spectrophotometric assay using BAEE (Nα-benzoyl-L-arginine ethyl ester) as a substrate for trypsin. The most common unit to express trypsin activity is the BAEE unit:

One BAEE unit of trypsin activity catalyzes a ΔA253 of 0.001 per minute with BAEE as substrate at pH 7.6 at 25 °C in a reaction volume of 3.20 mL.

Conversions: 1 BAEE unit = 1/3 USP unit

1 I.U. (International Unit) = 270 BAEE Units = 90 USP units

The designation 1:250 indicates that one part of trypsin digests 250 parts of casein (USP activity assay). Trypsin 1:250 has an activity of at least 250 USP units.

Please note: Even if the activity is generally noted by all manufacturers - for most applications the specific trypsin activity is practically irrelevant.

Related products

1500	Water sterile for cell biology
2098	D-PBS (10X) w/o Ca and Mg for cell biology
1429	D-PBS (1X) w/o Ca and Mg (pH 7.4) for cell biology
1346	D-PBS (1X) powder mixture w/o Ca and Mg for cell biology
1190	Phenol red for biochemistry
1444	Trypsin 0.05 % - EDTA (0.02 %) solution in HBSS w/o Ca and Mg, with Phenol red
4266	Trypsin inhibitor (1 mg/ml) for cell biology

JB12072021