

Trypsin derived from porcine pancreas, Trypsin / EDTA solutions

Trypsin/EDTA solutions (0.05 or 0.02 % in PBS, without Ca²⁺, Mg²⁺) are used for passaging mono-layer cultures. Trypsin affects the cell membrane and the cellular, and also the intercellular mucoproteins. Trypsin can be inhibited with the use of serum (see tab. 44, page 105).

The Trypsin dry substance applied for the production of the different Trypsin solutions will be tested for mycoplasma and parvoviruses. Only in case both results are negative, the solutions are released.

Product	Cat. No.	Unit
EDTA (Versen), 1 % in PBS without Ca ²⁺ , Mg ²⁺ Storage temperature: +2 °C – +8 °C	L 2113	100 ml
Trypsin (1:250), 0.25 % in PBS without Ca ²⁺ , Mg ²⁺ Storage temperature: –20 °C	L 2123	100 ml
Trypsin (1:250), 2.5 % in PBS without Ca ²⁺ , Mg ²⁺ Storage temperature: –20 °C	L 2133	100 ml
Trypsin (1:250)/EDTA (0.05/0.02 %) in PBS without Ca ²⁺ , Mg ²⁺ Storage temperature: –20 °C	L 2143	100 ml
(10x) Trypsin (1:250)/EDTA (0.5/0.2 %) in (10x) PBS without Ca ²⁺ , Mg ²⁺ Storage temperature: –20 °C	L 2153	100 ml
Trypsin (1:250)/EDTA (0.25 %/0.02 %) in PBS without Ca ²⁺ , Mg ²⁺ Storage temperature: –20 °C	L 2163	100 ml
Trypsin powder substance Trypsin activity is min. 1250 USP units/mg. The activity of Trypsin dry substance is about five times more than the activity of Trypsin (1:250)* Storage temperature: +2 °C – +8 °C	L 2103-20G	20 g

Specification of the used raw substance trypsin (1:250):

- water soluble at 2 % (w/v)
- loss on drying: < 5 %; ph value 3.0 – 4.0
- specific activity range: 275 USP U/mg (+/- 25 %)

Definitions USP:

- 1 USP unit catalyzes a change in absorbance of 0.003 per minute at 253 nm at +25 °C; pH 7.6 by the hydrolysis of BAEE (Benzoyl-L-arginine-ethylester).
- 1 USP unit=1 NF unit (National Formulary XIII pp. 646/7).

* In order to adapt the activity of this Trypsin dry substance to the activity of Trypsin 1:250, only 20 %, so 1/5, of the Trypsin dry substance with the activity of minimum 1250 USP units/mg is applied

Tab. 43: Composition of the Trypsin/EDTA preparations

Substance	EDTA (Versen) (in mg/l) Cat. No. L 2113	Trypsin (0.25 %) (in mg/l) Cat. No. L 2123	Trypsin (2.5 %) (in mg/l) Cat. No. L 2133	Trypsin/EDTA (0.05/0.02 %) (in mg/l) Cat. No. L 2153	(10x) Trypsin/ EDTA (in mg/l) Cat. No. L 2153
NaCl	8000	8000	8000	8000	80000
KCl	200	200	200	200	2000
Na ₂ HPO ₄	1150	1150	1150	1150	11500
KH ₂ PO ₄	200	200	200	200	2000
EDTA-Na ₂	10000	-	-	200	2000
Trypsin	-	2500	25000	500	5000

Recommendations Trypsin (1:250)/EDTA solutions:

Trypsin (1:250)/EDTA solutions (0.05/0.02 % in PBS without Ca²⁺, Mg²⁺) to passage monolayer cultures:

1. Wash cell culture (monolayer) using physiological saline (w/o Ca²⁺, Mg²⁺).
2. Apply a small quantity of Trypsin (1:250)/EDTA solution with a pipette to cell culture flask; a thin film is sufficient.
3. Incubate culture flask at +37 °C for approx. 5 min.
4. Centrifuge cell suspension or transfer directly to a serum containing culture medium.

With higher Trypsin concentrations, it is recommended to trypsinize at +2 – +8 °C.

Tab. 44: Comparison of Collagenase and Trypsin

Property	Collagenase	Trypsin
Site of action	collagen, reticulin and other intercellular substances	cellular and intercellular mucoproteins
pH optimum	6.5 – 7.8	7.8 – 8.7
Optimal activity	with Ca ²⁺ and Mg ²⁺	without Ca ²⁺ and Mg ²⁺
Impact on cells	no damage to cell membranes	damage to cell membranes
Effect of serum	Collagenase may be inhibited, depending on serum lot and Collagenase type, inhibition somewhat less as compared to Trypsin	loss of activity, inhibition