



User Manual of Trypelectase Recombinant Trypsin



Introduction of Trypsin recombinant trypsin

Trypsin is a serine protease that exists in the digestive system of many vertebrates and functions as a digestive enzyme. In the pancreas, it serves as a precursor for enzymes, and inactive trypsinogen is synthesized. The AA sequence of Trypsin recombinant trypsin is consistent with that of pancreatic trypsin derived from pigs, and is produced through recombinant expression in E. coli. It can replace traditional extraction of pancreatic enzymes for cell culture processes such as vaccines, stem cells, and immunotherapy, and can be used for digestion of adherent cells. Aprotinin and soybean trypsin inhibitors can inhibit their enzyme activity.

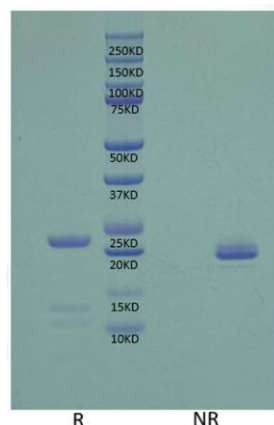
Characterization of Trypsin recombinant trypsin

Item	Description
Sequence Source	Pig
Appearance	White crystalline powder
Molecular weight	23.4kDa
Purity (RP-HPLC)	β -trypsin $\geq 70\%$, α -trypsin $\leq 20\%$
Specific activity	4608 (U/mg protein)
Endotoxin	≤ 5 EU/mg
Residual amount of exogenous DNA	≤ 100 pg/mg
Microbial limit	< 5 cfu/g
Optimal pH	6.8-7.3
Cofactor	Ca^{2+}
Storage conditions	-20°C

Activity unit: Define as 1 unit by 25°C , pH7.6, reaction system 3.2ml (1cm optical path), Enzymatic hydrolysis of BAE per minute increases the absorption value at 253nm by 0.003

Trypsin recombinant trypsin SDS-PAGE

Sample size: 5 μg



Non reducing and reducing SDS-PAGE showed a protein molecular weight of approximately 23 kDa

Trypsin recombinant trypsin is mainly used for cell dissociation:

- (1) Obtaining primary cells for digestion and dissociation of tissue
- (2) Used for passage digestion of adherent cells
- (3) Cell digestion for microcarrier culture



Method of using adherent cell digestion:

Reagents and consumables: pancreatic enzyme dry powder, solution, PBS (pH7.4), 0.22μm filter membrane

1. Remove Trypsinase dry powder reagent bottle from the refrigerator and restore it to room temperature (about 30 minutes)
2. Dissolved solution: 1mM HCl, 5% sorbitol, 10mM CaCl₂ or 1mM HCl, 5% sorbitol, pH 3.0, with 0.22μm filter membrane filtration for sterilization.
3. Weigh the dry powder and gently re dissolve it with a solution to achieve a trypsin concentration of 10mg/ml
4. Preparation of cell digestion Trypsinase enzyme buffer:
Mix the mother solution with sterile PBS (pH7.4) 1:20 (v/v) to form a 0.5mg/mL pancreatic enzyme digestion solution. If incubation at 37 °C is required, PBS can be placed at 37 °C in advance for incubation before diluting the mother solution.

Storage and Validity

Dry Powder: Trypsinase recombinant trypsin freeze-dried powder was stored at -20~-80 °C and remained stable for 24 months;

Mother Solution: After dissolving with 1mM HCl, 5% sorbitol, 10mM CaCl₂ or 1mM HCl, 5% sorbitol, it can be stored at -20 °C for six months, with no more than 8 repeated freeze-thaw cycles.

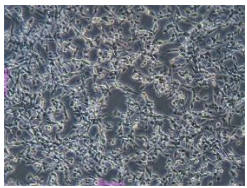
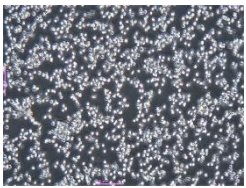
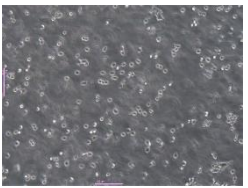
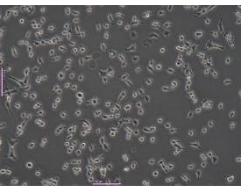
Cell Digestion Experiment:

Digest and passage the cells with T25 after cell fusion reaches over 90%. Remove the cell culture medium and wash once with 10mL PBSS. Afterwards, add 0.5mL 0.5mg/mL (1x PBS dissolved) of pancreatic enzyme digestion solution to each bottle, let it digest at 37 °C for 3 minutes, neutralize pancreatic enzyme with 4.5mL of complete culture medium, and gently disperse cells using a pipette. Use a cell counter to detect cell count and activity. Pass 0.33×10^6 cells into T25 culture bottles. Observe the cell adhesion after 24 hours of passage.

The relevant cell digestion data are as follows:

Cell	Activity	Concentration	Digestion time
CT26.WT	98%	0.05% (m/V)	3min±0.04
HEK293FT	97%	0.05% (m/V)	3min±0.04

The process of digesting cells using Trypsinase recombinant trypsin and the 24-hour cell adhesion image after passage

Cell	Before digestion	Digestion for 3min	After neutralization	24-hour cell adhesion after passage
CT26.WT				
HEK293FT	