

Instructions for using TrypLUS enzyme

Usage of TrypLUS Enzyme

Used for cell dissociation in adherent culture

1. Obtaining primary cells for dissociation and dissociation of tissue blocks
2. Used for passage dissociation of adherent cells
3. Cell dissociation for microcarrier culture

Storage conditions and expiration date

Storage conditions: 4°C Dark storage, stable for 24 months

Brief Introduction of TrypLUS Enzyme

TrypLUS (EC: 3.4.21.4) is a trypsin like protease derived from *Fusarium oxysporum*, expressed and purified from *Escherichia coli*. This enzyme has the same cleavage site and pH activity range as trypsin, and can cleave the carboxyl end of arginine and lysine in the protein sequence.

TrypLUS enzyme has been proven to effectively dissociate adherent mammalian cell lines in cell experiments, exhibiting dissociation kinetics similar to porcine trypsin and exhibiting low cytotoxicity. Cell replication, proliferation kinetics, and long-term maintenance can be consistent with cells harvested by animal trypsin. Purity analysis shows that TrypLUS enzyme has higher purity, more stable molecules, and better activity maintenance compared to traditional trypsin.



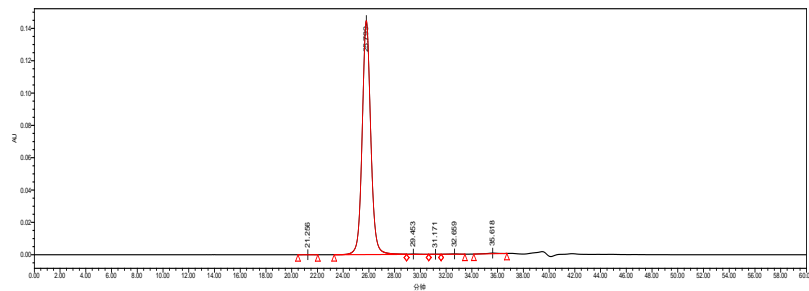
P1 . TrypLUS 3D Structural Diagram

Production Information of TrypLUS Enzyme

Table1 . TrypLUS Enzyme release testing items and results

Item	Description
Origin of Sequence	<i>Fusarium oxysporum</i>
Appearance	Transparent
molecular weight	22.2 kDa
Purity(SEC)	≥95%
Specific activity*	≥27,000 U/mg
Bacterial endotoxin	< 5 EU/ml
Host protein residue	< 10 PPM
Optimum pH	7.0-7.4
Storage temperature	2-8°C

* Definition of enzyme activity unit: 25 °C, pH 7.6, reaction system 3.2 mL (1cm optical path), enzymatic hydrolysis of BAEE (N-Benzoyl-L-Arginine-Ethyl ester) per minute increases the absorption value of 253nm wavelength by 0.003, defined as one activity unit(Unit/mg protein) (2020 edition of Chinese Pharmacopoeia)



P2. SEC-HPLC enzyme purity detection (purity of 99.28%)

Instruction for dissociation of adherent cells

1. TryPLUS can be used at room temperature or pre heated to 37°C before use to achieve good dissociation effect.
2. Operation steps(T25 bottle as an example. If for T75 bottle,the recommended dosage of TryPlus dissociation solution is 5ml)

2.1 After the cells cultured in T25 bottles reach over 90% fusion, they are then dissociated and passaged

2.2 Remove the cell culture medium and wash with 10mL PBS.

2.3 Add 1.7mL of TryPLUS enzyme to each bottle and dissociate at 37 °C for 3 minutes.

2.4 Resuspend the cells with 8.3mL complete medium, and gently blow away the cells with Pipette.

2.5 Use a cell counter to measure cell count and viability.

2.6 Add 1×10^6 cells were passaged into T25 culture bottles.

Observe the cell adhesion after passage for 24 hours.

Note: No need to use pancreatic enzyme inhibitors after dissociation

Experimental flow:

