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# Recombinant Trypsin (Cell Culture Grade)

**Product Number: RPJ06**

## Shipping and Storage

Storage at -15℃ and below, away from light, sealed.

## Description

Trypsin is a serine protease that selectively hydrolyzes peptide chains composed of the carboxyl terminus of lysine or arginine in proteins. This product is a recombinant trypsin powder isolated and purified from recombinant Pichia pastoris, and then freeze-dried.

This product belongs to the genetic engineering enzyme of biochemical grade and does not contain enzyme inhibitors such as DFP, PMSF, and TLCK. It has the same activity and specificity as natural trypsin. The activity of this product is inhibited by serine protease inhibitors such as PMSF and TLCK, as well as metal ion chelating agents such as EDTA.

## Application

1. Used for the production of biopharmaceuticals, such as insulin and GLP-1 drugs.
2. Research on protein structure and function, such as protein mass spectrometry, sequencing, and peptide mapping analysis.

## Product advantages

1. No animal origin: no animal viruses, no pathogenic substances, no external factor contamination, high safety.
2. High purity: high specific activity; The residual host protein is less than the limit requirement for biological products.
3. Complies with the standards of the Chinese Pharmacopoeia 2020 edition and USP43.
4. Production scale of over 1000L.
5. Compliance: The production equipment and environment comply with relevant regulatory requirements and GMP guidelines.
6. Complete quality documents: Relevant regulatory support documents can be provided according to customer requirements.

## Features

| Project | Feature |
|---|---|
| Appearance | White or off white freeze-dried powder |
| Molecular weight | 23.5kD±2.4kD |
| Purity (SDS-PAGE) | ≥95% |
| Purity (HPLC) | α- trypsin should not exceed 20%<br>β- trypsin must not be less than 70% |
| Specific activity | ≥3800USP unit/mg pro |
| Protein content | Not less than 40% |
| Residual amount of exogenous DNA | ≤100ng/mg pro |
| Residual amount of bacterial protein | ≤100ng/mg pro |
| Optimal pH | 7.0~9.0 |
| Solubility | Soluble in water or buffer solution |

Enzyme activity definition: 25℃, pH7.6, The reaction system is 3.0mL (1cm optical path), and the enzymatic hydrolysis of BAEE increases the absorption value at 253nm by 0.003 per minute, defined as one USP unit.

## Protocol

**1. Protease digestion usage method**

1.1. Protein preparation - Dissolve the protein in a solution of 25mM Tris HCl at pH 8.0.

1.2. Enzyme preparation - Weigh the required enzyme powder and dissolve it in 1mM HCl to achieve a concentration of 1-10mg/ml.

1.3. Reaction conditions - enzyme quantity: target protein (mass ratio=1:50~1:1000, optimal pH is 7.0~9.0).

**2. Cell dissociation**

2.1. Solution preparation: Dissolve recombinant trypsin at room temperature, take an appropriate amount of trypsin, add HBSS equilibrium solution (or other suitable buffer for cell digestion, if EDTA is needed, the recommended final concentration is 0-1mM, preferably not exceeding 2mM), and the recommended concentration of trypsin for general use is about 0.1-0.3mg/ml (concentration adjusted according to different cells), gently mix well; This step can be operated at room temperature;

2.2. Filter the trypsin solution using a 0.22μm filter membrane and transfer it to a sterile container; This step can be operated at room temperature;

2.3. The filtered trypsin solution should be used directly on the same day as required (e.g. adding 1ml to T25 bottle, digesting cells at 37 ℃).

## Transportation and storage stability

1. Transportation stability: Ice pack insulation transportation, stable activity.
2. Storage stability: Freeze dried powder can be stored stably for at least 24 months at -15℃ and below. Dissolve 1mM hydrochloric acid or 50mM acetic acid and store at -15℃ or below. Repeat freezing and thawing 10 times without any loss of activity. It is recommended to store at 2-8℃ after dissolution for no more than 7 days, and at -15℃ and below after dissolution for no more than 30 days.

## Note

1. This product has hygroscopicity and should be taken after equilibration at room temperature.
2. This product should not be used for clinical diagnosis and treatment.

Linearization vector sequence (green and purple indicate upstream and downstream homologous arm regions, bold indicates enzyme cleavage site sequence):


The design for inserting fragment amplification primers is (blue and pink indicate overlapping sequences between fragments):

Fragment1 :

Primer F: 5′-gaggcttacgcagcaggt+AAGCTT(optional)+**tgccagtggcgata**-3′

Primer R: 5′-taactca...acacgag-3′

Fragment2 :

Primer F: 5′-ctcgtgt...tgagttattagtttcaacgctg-3′

Primer R: 5′-ttaacta...tgcatag-3′

Fragment3 :

Primer F: 5′-ctatgca...tagttaacggtcaccgtgctggtat-3′

Primer R: 5′-gctcgggtagatcgtcttga+GAATTC(optional)+**ctgcgccttatccggtaa**-3′

### 2.4. PCR amplification of exogenous DNA fragments

Suggest using high fidelity DNA polymerase for amplification of exogenous DNA fragments; There is no need to consider whether there is an A tail at the end of the product, which does not affect the final splicing accuracy and efficiency. Similar to the process of carrier preparation, if the PCR amplification band of exogenous DNA is single, purification is not necessary and it can be directly used for subsequent splicing reactions. When the purity of PCR amplification products is low, it is recommended to perform gel recovery purification, which is beneficial for improving recombination efficiency.

Note: If the template used for preparing exogenous DNA is a circular plasmid with the same resistance as the subsequent vector to be spliced, the residual exogenous plasmid template will cause a large amount of transformation background, affecting the selection of target clones, and therefore the plasmid template needs to be removed; The target fragment can

be recovered by electrophoresis or the circular plasmid with methylation modification can be removed by Dpn I enzyme digestion.

**2.5. Recombination reaction**

Prepare the reaction system on ice, react at 37℃ for 5-30 minutes (set 1-2 insertion fragments for 5-15 minutes); 3-5 insertion segments set for 15-30 minutes. After the reaction is complete, place the reaction product on ice for 5 minutes to terminate the reaction. The reaction products can be directly used for conversion experiments or frozen at -20℃ for future use.

| Component | Volume |
|---|---|
| 2×fast Multi One-Step Cloning kit | 10μL |
| Linearized vector | 20ng/kb (minimum 40ng) |
| Exogenous DNA | Insert fragments at 40ng/kb (minimum 20ng/fragment) |
| $ddH_2O$ | Up to 20μL |

**Note:** Suggest setting up a negative control, that is, the above system does not add 2×fast Multi CloneMix; If many clones grow from the negative control, it indicates that there are problems such as incomplete linearization of the vector or incomplete removal of the template plasmid for exogenous fragment amplification during the preparation of the vector or exogenous insertion fragment, and it needs to be re prepared or purified.

The optimal molar ratio of the recommended carrier and exogenous insertion fragment is n (carrier): n (insertion fragment)=1:2; It is recommended to calculate the dosage directly based on the fragment length without calculating the molar concentration. Taking a 4kb vector as an example, the dosage is 20ng/kb×4kb=80ng, and the minimum dosage for vectors below 2kb is 40ng;

Taking a 1kb insertion fragment as an example, the usage amount is 40ng/kb×1kb=40ng. For exogenous insertion fragments below 0.5kb, the minimum usage amount is 20ng. It is recommended to use instruments with precise temperature control, such as PCR machines, for the reaction. Differences in reaction temperature or duration can greatly affect the efficiency of cloning.

**2.6. Recombinant product conversion**

Thawing clones on ice using competent cells (such as DH5 α competent cells).

Take 10μL of the above reaction solution and add it to 100μL of competent cells. Gently mix well and let it stand on ice for 30 minutes.

After being heated in a 42℃ water bath for 90 seconds, immediately place it on ice to cool for 2-5 minutes.

Add 900μL of SOC or LB liquid culture medium (without antibiotics) and shake at 37℃ for 45 minutes to rejuvenate.

Centrifuge the rejuvenated bacterial solution at 5000 rpm for 5 minutes, discard 800μL of supernatant, and suspend the bacterial body with the remaining culture medium. Use a sterile coating rod to coat the bacterial solution on a plate containing the correct resistance, and invert it in a 37℃ incubator for 12-16 hours.

The clones grown on the tablet can be quickly identified using the "colony PCR method". For positive clones, further sequence determination can be carried out according to experimental requirements.

**Note:** The maximum conversion volume of recombinant products should not exceed 1/10 of the volume of competent cells used.

**2.7. Positive clone identification**

2.5.1. Colony/bacterial liquid PCR identification: Use a pipette to pick up a single colony and mix it with 10μL of sterile water, then take 1μL as a template, or directly dip the colony into the PCR system for amplification (it is recommended to use at least one universal primer to avoid false positive results);

2.5.2. PCR identification using plasmids as templates: Select monoclonal antibodies into LB medium containing corresponding antibiotics, shake overnight at 37℃ and 220 rpm, extract plasmids as templates, and amplify using vector universal primers or specific primers;

2.5.3. Enzyme digestion identification (if necessary): Select monoclonal antibodies into LB medium containing corresponding antibiotics, shake overnight at 37℃ and 220 rpm, extract plasmids, use corresponding

endonucleases to cleave plasmids, and detect fragment size by electrophoresis;

2.5.4. Sequencing: It is recommended to use vector universal primers for sequencing and identification.

**Note**

1. When enzyme digestion is used to prepare linearized vectors, in most cases, there is no need to recover the vector. However, for larger exogenous templates, recovering the vector can improve cloning efficiency and positivity rate.
2. Partial restriction endonucleases produce sticky ends, and their linearized carriers can be directly transformed into E. coli cells for repair, resulting in false positive clones. In response to this situation, it is recommended to replace the enzyme, perform double enzyme digestion, or increase the number of validated clones.
3. When preparing linearized vectors or exogenous fragments by reverse PCR amplification, if the PCR product is single, it can be directly used for subsequent recombination reactions. If the PCR product is not single, it should be purified by nucleic acid electrophoresis with gel recovery; When preparing linearized vectors by reverse PCR amplification, circular plasmids are used as templates, and the PCR products need to be treated with Dpn I to reduce the impact of residual circular plasmid templates on the cloning positivity rate.
4. Due to the presence of salt ions in the recombination reaction system, the reaction products cannot be directly electrocuted. If electric shock conversion is required, desalination treatment of the reaction products is necessary. Due to the limited amount of DNA used for the reaction, it is not recommended to use ethanol precipitation or gel to recover pure DNA
5. The desalination process can be carried out using a filter membrane suitable for micro DNA dialysis.
6. When directly using enzyme digestion products or PCR amplification products for recombination reactions, it is recommended to add no more than 1/5 of the total volume. If the exogenous DNA fragment is obtained by PCR amplification and directly used for recombination reaction without recovery and purification, it is also recommended that the reaction volume should not exceed 1/5 of the total volume. If neither the exogenous DNA fragment nor the linear vector is purified, the sum of their volumes should be less than 1/5 of the total volume.
7. In order to achieve higher cloning efficiency and positivity rate during the recombination reaction, it is recommended that the molar ratio of linear vector to exogenous fragment be 1:2.
8. This product is for scientific research use only.