APB070Hu02 100μg
Active Tryptase (TPS)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Ile31~Pro275
Tags: N-terminal His-tag
Purity: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose .
Original Concentration: 200μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 6.8
Predicted Molecular Mass: 30.7kDa
Accurate Molecular Mass: 33kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.
Store at 2-8ºC for one month.
Aliquot and store at -80ºC for 12 months.

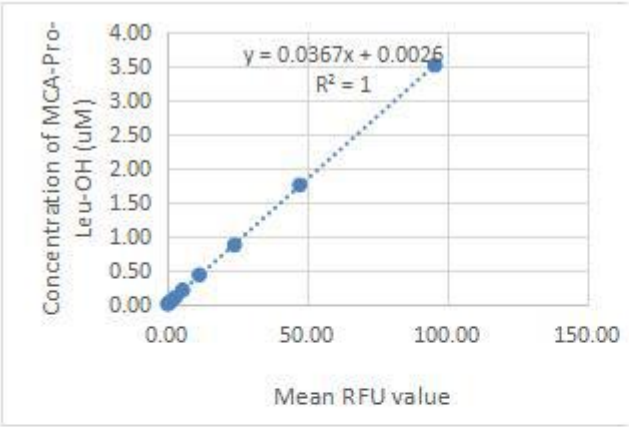
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37ºC for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

IVGGQEAPRSKWPWQVSLRVHGPYWMHFCGGSLIHPQWVLTAAHCVGPDVKDLAALRVQLREQHLYYQDQLLPVSRIIVHPQFYTAQIG
ADIALLELEEPVNVSSHVHTVTLPPASETFPPGMPCWVTGWGDVDNDERLPPPFPLKQVKVPIMENHICDAKYHLGAYTGDDVRIVRDD
MLCAGNTRRDSCQGDSGGPLVCKVNGTWLQAGVVSWGEGCAQPNRPGIYTRVTYYLDWIHHYVPKKP

[ACTIVITY]

Tryptase is a serine protease with trypsin-like activity, which is sometimes also referred to as Mast Cell Protease 7. It is stored in the secretory granules of mouse mast cells. It exhibits anticoagulant activity due to its ability to degrade fibrinogen in the presence of the diverse array of protease inhibitors in plasma. The activity of recombinant human TPS is measured by its ability to cleave a fluorogenic peptide substrate Mca-Arg-Pro-Lys-Pro-Val-Glu-Nval-Trp-Arg-Lys(Dnp)-NH2 in the assay buffer 50 mM Tris, pH 8.5. The rhTPS is diluted to 200 ug/ml in 50 mM Tris, 150 mM NaCl, 10 mM CaCl2, 0.05% (w/v) Brij-35, pH 7.5, then activated with 0.1 ug/ml Thermolysin at 37 ℃ for 15min followed by adding 10 mM 1, 10 phenanthroline to stop activation. The activated rhTPS is diluted to 50 ug/mL in heparin incubation buffer of 100 μg/mL heparin, 50 mM MES, pH 5.5 and incubated at room temperature for 2 hours. Then the rhTPS was diluted to 12.5 ug/ml in assay buffer and load into a black well plate 50 μL and start the reaction by adding 50 μL of 20 μM substrate, with a substrate blank containing 50 μL assay buffer, 50 μL substrate, and no rhTPS. Then read at excitiation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes. The specific activity of recombinant human TPS is > 25 pmol/min/μg.



RFU (320/405)	MCA-Pro-Leu-OH (product) uM
95.78	3.52
47.46	1.76
24.20	0.88
11.63	0.44
5.71	0.22
3.05	0.11
1.52	0.05
0.77	0.03

Figure 1. The standard curve of MCA-Pro-Leu-OH

Specific Activity (pmol/min/μg) =

$$\frac{\text{Adjusted Vmax * (RFU/min) x Conversion Factor ** (pmol/RFU)}}{\text{amount of enzyme (ug)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH

[IDENTIFICATION]

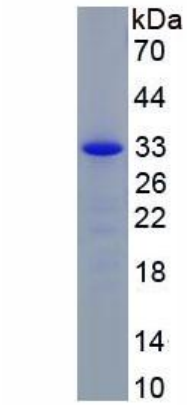


Figure 2. SDS-PAGE

Sample: Active recombinant TPS, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.