Tribo™ Tryptase Activity Assay (Catalog# TBS2101)

DESCRIPTION

Tryptase, a tetrameric serine proteinase, has emerged as the major component of mast cell granules, comprising up to 20% of the total protein of mast cells derived from lung, colon, and skin tissue. Because it is stored almost exclusively in mast cells, tryptase is a popular indicator of mast cell activation and a target for therapeutic intervention in allergic diseases.

The Tribio™ Tryptase Activity Assay Kit provides a quick, efficient, and sensitive system for evaluation of tryptase activity in cell lysates, supernatants or for inhibitor screening.

APPLICATION

- Cell culture supernatants, cell lysates or other tryptase-containing samples.
- Testing of purified tryptase enzyme, in vitro inhibitor screening.
- Cell degranulation.

FEATURES

- Sensitive and accurate: The Kit detects as low as 10µM in solution.
- Simple and high-throughput: The procedure is easily adapted to automation with no separation required as a high-throughput assay.

KIT CONTENTS (100 assay)

Component	Amount
5x Assay Buffer	**10 mL**
Tryptase inhibitor	**200 µL**
Tryptase Substrate	**2 mL**
pNA Standard	**100 µL (10 mM)**
Calcium Ionophore	**50 µL(1mM)**
Tryptase Positive Control	**200 µL (10 µg/ mL)**

Storage conditions: Store the kit at –20°C, protected from light. Shelf life: 6 months.

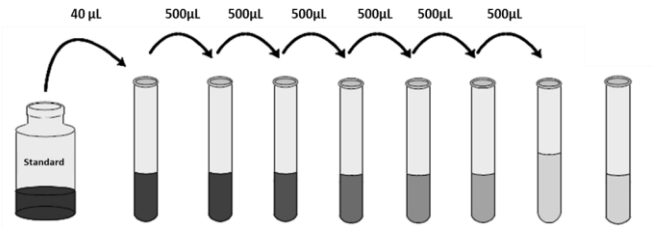
PROCEDURES

Reagent Preparation:

1. Tryptase Positive Control: Thaw vial at 2-8°C. Aliquot and store at -20°C up to the vial's expiration date. Avoid multiple freeze/thaw cycles.
2. 5X Assay Buffer: Dilute the 5X Assay Buffer to 1x working buffer with deionized water.
3. Tryptase Inhibitor: Thaw vial at 2-8°C. Aliquot and store at -20°C up to the vial's expiration date. Avoid multiple freeze/thaw cycles.
4. pNA Standard Preparation as below Diagram:

a. Label 1.5mL tube from Std1 to 8. As below the diagram.
b. Add 960 µL of 1x Assay Buffer to Std1, and 500 µL to Std2 to 8.
c. Take 40 µL of 10mM pNA Standard Stock solution to Std1, and fully mix, then add 500µL of Std1 to Std2, then make 2x series dilution in Std3 through 7. Std8 is 1x Assay Buffer alone as a standard 0. The standard concentration range is 400, 200, 100, 50, 25, 12.5, 6.25µM, and 0.

Diagram for Tryptase Standard Preparation



Standard	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	960	500	500	500	500	500	500	500
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Higher Std (µL)	40	500	500	500	500	500	500	
Final Conc (µM)	400	200	100	50	25	12.5	6.25	0

5. Tryptase Positive Control: **A)** Make a 1.0 µg/mL Tryptase Positive Control by diluting the 10.0 µg/mL stock solution in 1X Assay Buffer; **B)** add 180 µL of the Positive Control (1.0 µg/mL) to the wells as a positive control; **C)** Finally, add 20 µL of reconstituted Tryptase Substrate (see **Assay Procedures**) to each well. Mix well and incubate 1-2 hrs at 37°C. Note: Assay incubation times may be extended for higher sensitivity. **D)** Read OD at 405 nm in a microplate reader.
6. Calcium Ionophore: The Calcium Ionophore should be pre-diluted in DMSO prior to usage (recommended final concentration of 1.0 - 500 nM). *Note: For best stability, only dilute the required volume of Calcium Ionophore; retain the rest as stock solution.*
7. Tryptase Inhibitor: The Tryptase Inhibitor may be diluted in 1X Assay Buffer prior to usage (recommended final concentration of 1.0 - 100 µM). *Note: For best stability, only dilute the required volume of Tryptase Inhibitor; retain the rest as stock solution.*

Preparation of Mast Cell Samples:

8. Samples are isolated and prepared from areas known to contain a high percentage of mast cells with suitable isolation techniques.
9. After isolating the cells, wash the cells with 1X Assay Buffer. Remove the solution, and resuspend the cells with 1X Assay Buffer.
10. Count and adjust the cell concentration to 1.0 – 10.0 x 10^6 cells/mL with 1X Assay Buffer.

11. Add 1.0 mL of the cell suspension to a microcentrifuge tube.
12. For treatment with calcium ionophore, add 10 µL of solution (see Preparation of Reagents Section) to the cell suspension (recommended final concentration of 1.0 – 500 nM).
13. For treatment with tryptase inhibitor, add 10 µL of solution (see Preparation of Reagents Section) to the cell suspension (recommended final concentration of 1.0 – 100 µM).
14. Incubate the cells in a 37°C, 5% CO_2 incubator for 60 minutes.
15. Collection of tryptase sample:
    A. Supernatant: Centrifuge the cell suspension at 700 x g. Carefully collect the supernatant, leaving the cell pellet. Store at 2° to 8°C.
    B. Lysate: Centrifuge the cell suspension at 700 x g. Carefully aspirate the supernatant and discard. Wash cell pellet once with cold PBS. Centrifuge and discard supernatant. Resuspend the pellet in 1mL of 1X Assay Buffer. Sonicate the suspension with a pulse sonicator until cells are thoroughly lysed. Centrifuge down the cell debris, collecting the lysate sample. Store at 2° to 8°C.

ASSAY PROCEDURES

1. Prepare assay mixture in a 96-well microliter plate or standard microcentrifuge tubes, according to the following table as a guide for preparing and assay samples and controls.

Sample	Assay Mixture			Tryptase Substrate	Total Volume
	Tryptase Sample	Inhibitor	1X Assay Buffer		
Buffer Blank	0µL	0µL	200µL	0µL	200µL
Substrate Blank	0µL	0µL	180µL	20µL	200µL
Standard	0µL	0µL	0µL	0µL	200µL
Test Sample	180µL or X	0µL	(180-X) µL	20µL	200µL
Sample+ Inhibitor (optional)	180uL or X	YµL	180-(X+Y) µL	20µL	200µL

X = volume of cell lysate sample added if less than 180 µL.
Y = volume of inhibitor added.

2. Initiate the colorimetric reaction by adding 20 µL of the Tryptase Substrate to each test and control well *(Note: DO NOT add the substrate to standard wells).*
3. Incubate samples for 1-2 hours at 37°C (**Note**: *Assay incubation times may be extended for higher sensitivity; the recommendation time is 90 mins).*
4. Read OD at 405 nm in a microplate reader (**Note:** *Background reading from cell lysates, supernatants and buffers should be subtracted from the readings before calculating fold increase in tryptase activity*).

CALCULATION OF RESULTS

The following chart illustrates typical results including dilutions of the *p*NA Standard (Fig.1) and the activity curve of the Tryptase Positive Control (Fig.2) in the kit. Optical Density (OD) values obtained with the Assay Kit may be compared with known standards or other test samples to obtain relative activities. One should use the data below for reference only.

Note: pH value of assay buffer effects on the tryptase activity displayed in Fig.3. The kit assay buffer was optimized to achieve best result.

Figure1: pNA Standard Curve

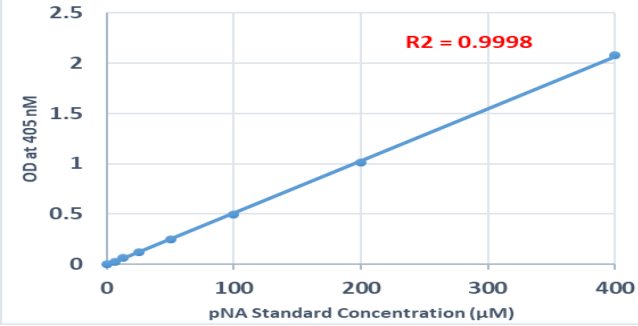


Figure 2

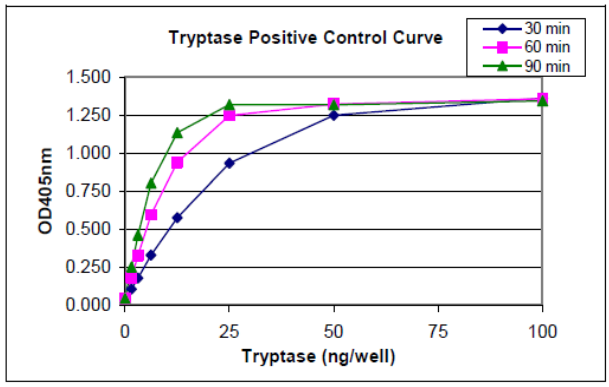
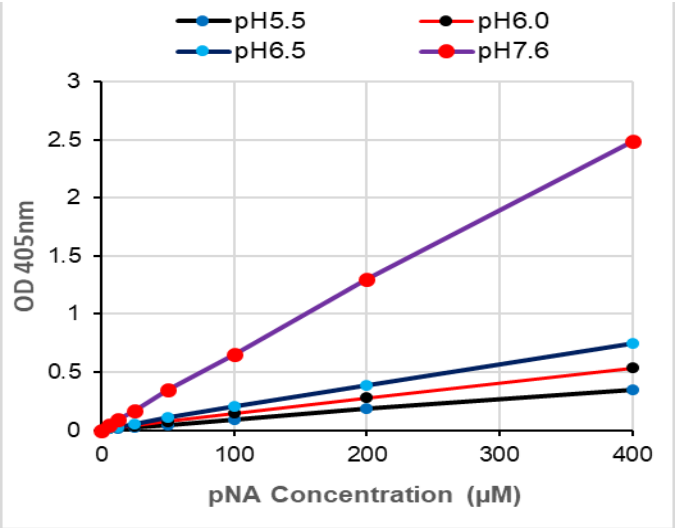


Fig.3: Effects of pH Value on Tryptase Activity



REFERENCES

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RELATED PRODUCTS

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Cytochrome C Oxidase Activity Assay (TBS2115)
Fast Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
Glucose Oxidase Activity Colorimetric/Fluorometric Assay (TBS2088)
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Protein Assay Kits (TBS2005)
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