Mast Granulation Assay Datasheet

Version 2.0
Released Date: September 26th, 2025

Cat. No. G7800

Store at -20°C, protect from light.
Shelf life: 6 months.

Product Description

Fast. Reliable. Insightful.

Granulation is a defining feature of mast cells, essential for their ability to rapidly respond to environmental threats and actively participate in immune responses. Without granulation, mast cells would be functionally impaired—highlighting the importance of accurately assessing this process.

To meet this need, our **Mast Granulation Assay**, based on **Tryptase** activity detection, offers a powerful solution. Among the various assays available, it stands out for its **simplicity, reproducibility, and sensitivity**.

Applications

- A **reliable biomarker** for assessing mast cell presence and functionality
- A **valuable tool** for **inhibitor screening** in anti-inflammatory and anti-allergic drug development

Product Component	Quantity	Part No.
5X Assay Buffer	10 mL	G7800-1
Tryptase inhibitor	200 µL (10 mM)	G7800-2
Tryptase Substrate	2 mL	G7800-3
pNA Standard	400 µL (10 mM)	G7800-4
Calcium Ionophore	50 µL (1 mM)	G7800-5
Positive Control	200 µL	G7800-6

Procedures

Reagent Preparation

1. **Positive Control**
   - Thaw the vial at 2–8°C.
   - Aliquot and store at –20°C until the vial's expiration date.
   - Avoid multiple freeze/thaw cycles.

2. **5X Assay Buffer**
   - Dilute the 5X Assay Buffer with deionized water to prepare a 1X working solution.

3. **Tryptase Inhibitor**
   - Thaw the vial at 2–8°C.
   - Aliquot and store at –20°C until the vial's expiration date.
   - Avoid multiple freeze/thaw cycles.

4. **pNA Standard Preparation (Refer to Fig. 1)**

   A. Label eight 1.5 mL tubes as Std1 through Std8.

   B. Add:
      - 480 µL of 1X Assay Buffer to Std1
      - 250 µL to each of Std2 through Std8

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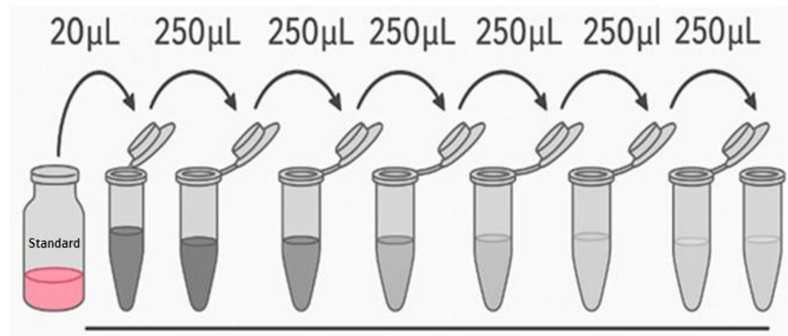

C. Add 20 μL of 10 mM pNA standard stock solution to Std1 and mix thoroughly.
- Transfer 250 μL from Std1 to Std2, mix well.
- Continue serial 1:2 dilutions from Std2 through Std7 by transferring 250 μL from one tube to the next.
- Std8 should contain only 1X Assay Buffer and serves as the blank (0 μM).

D. The resulting standard concentrations are:
- 400, 200, 100, 50, 25, 12.5, 6.25, and 0 μM

Fig.1 Diagram for Tryptase Standard Preparation

Standard	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (μL)	480	250	250	250	250	250	250	250
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	-
Addition (μL)	20	250	250	250	250	250	250	-
Final Conc. (μM)	400	200	100	50	25	12.5	6.25	0

5. **Calcium Ionophore**
   - Dilute the **Calcium Ionophore** in **DMSO** prior to use.
   - Recommended final concentration: 1.0–500 nM.

   Note: For optimal stability, prepare **only the amount needed for immediate use**. Store the remaining reagent as a **concentrated stock solution**.

6. **Tryptase Inhibitor (Working Dilution)**
   - The **Tryptase Inhibitor** may be diluted in **1X Assay Buffer** before use.
   - Recommended final concentration: **1.0–100 μM.**

   Note: To maintain stability, **dilute only the required volume**. Keep the **remaining stock undiluted**.

Preparation of Mast Cell Samples

1. **Mast Cell Isolation**
   - Isolate mast cells using an **appropriate method** to ensure **high purity**.

2. **Washing and Resuspension**
   - Wash the isolated cells with **1X Assay Buffer**.
   - Remove the supernatant and resuspend the cells in **fresh 1X Assay Buffer**.

3. **Cell Count Adjustment**
   - Count the cells and adjust the concentration to **1.0–10.0 × 10^6 cells/mL** using **1X Assay Buffer**.

4. **Aliquoting**
   - Dispense **0.5 mL** of the prepared cell suspension into a **24 well plate**.

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5. **Calcium Ionophore Treatment**
   - Add **10 µL** of the **prepared Calcium Ionophore solution** (refer to *Reagent Preparation* section).
   - Final concentration should be within **1.0–500 nM**.

6. **Tryptase Inhibitor Treatment (Optional)**
   - Add **10 µL** of the **prepared Tryptase Inhibitor solution** (refer to *Reagent Preparation* section).
   - Final concentration should be within **1.0–100 µM**.

7. **Incubation**
   - Incubate the treated cells at **37°C with 5% CO_2** for **60 minutes**.

Collection of Tryptase Sample

A. Supernatant Collection

- Collect and centrifuge the cell suspension at **700 × g**.
- Carefully collect the **supernatant** — this is the **supernatant sample**.
- Store the supernatant at **2–8°C** until testing.

B. Cell Lysate Preparation

- Resuspend the **cell pellet** from step A in **1X cold PBS buffer**.
- Centrifuge at **700 × g**, discard the supernatant.
- Wash the cell pellet again with **1X cold PBS**, centrifuge, and discard the supernatant.
- Resuspend the pellet in **0.5 mL of 1X Assay Buffer**.
- Sonicate the suspension using a **pulse sonicator** until cells are fully lysed.
- Centrifuge the lysate to remove debris.
- Collect the **supernatant** — this is the **cell lysate sample**.
- Store at **2–8°C** until testing.

Assay Procedures

1. **Assay Setup**
   - Prepare the **assay mixture** in a **96-well microplate** or **standard microcentrifuge tubes**.
   - Use the table below (not provided here) as a guide to set up **test samples** and **controls**.

Sample	Assay Mixture			Tryptase Substrate	Total Volume
	Tryptase Sample	Inhibitor	1X Assay Buffer		
Buffer Blank	0 µL	0 µL	200 µL	0 µL	200 µL
Substrate Blank	0 µL	0 µL	180 µL	20 µL	200 µL
Standard	0 µL	0 µL	0 µL	0 µL	200 µL
Test Sample	180 µL or X	0 µL	(180-X) µL	20 µL	200 µL
Positive control	20 µL	0 µL	160 µL	20 µL	200 µL
Sample+ Inhibitor (optional)	180 µL or X	Y µL	180-(X+Y) µL	20 µL	200 µL

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

2. **Initiate Colorimetric Reaction**
   - Add **20 µL of the Tryptase Substrate** to each **test and control well**.

     ⚠ **Note:** *Do NOT add substrate to standard wells.*

3. **Incubation**
   - Incubate the plate at **37°C for 1–2 hours**.

     ▯ **Recommended incubation time:** *90 minutes for optimal sensitivity.*
     ⚠ **Note:** Longer incubation may enhance assay sensitivity.

4. **Measurement**
   - Read the absorbance at **405 nm** using a **microplate reader**.

     Note: Subtract background absorbance (from cell lysates, supernatants, and buffers) **before** calculating fold increases in tryptase activity.

Calculation of Results

- The chart in **Figure 2** illustrates typical OD values resulting from serial dilutions of the **pNA Standard** included in this kit.
- **Optical Density (OD)** readings from test samples can be compared against:
  - The **pNA standard curve** for quantifying activity
  - Other **test samples or known controls** for determining **relative tryptase activity**

Note: Use the sample data in Figure 2 for reference only. Actual values may vary between runs and labs.

⚠ Important Considerations

- The **pH of the Assay Buffer** has a significant impact on observed **tryptase activity**.
- See **Figure 3** for a graphical representation of how different pH levels affect enzymatic activity.

✓ The assay buffer provided in this kit has been **optimized** to ensure the **best performance** under standard conditions.

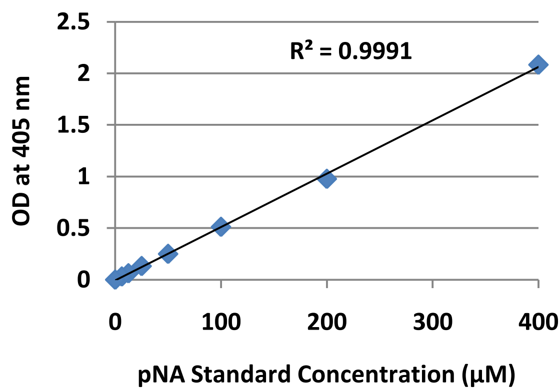

Fig. 2: pNA Standard Curve

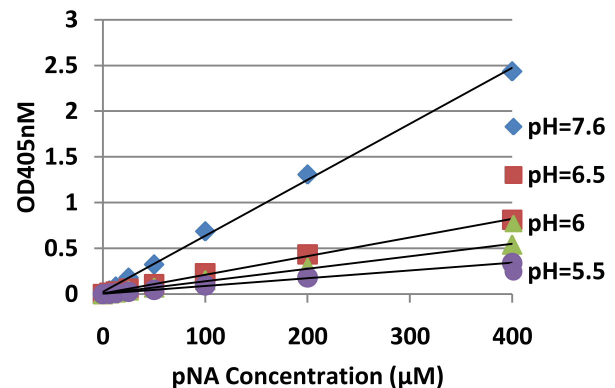

Fig.3. Effect of pH value on the kit result