Mast Cell Preparation for Granulation Assay – Guideline

Version 1
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Purpose:

To prepare **LAD2/LADR cells** for the **Mast Granulation Assay** *(G7800)* by sensitization with human IgE *(Y70000)* and crosslinking with anti-IgE *(Y79000).*

Materials:

Cat. No.	Product
T8157/ T8156	LAD2/ LADR cells
Y70000	Human IgE
Y79000	Human anti-IgE

Additionally you will require:

- hSCF-free culture medium *(complete medium)*
- hSCF-free medium + 0.5% BSA *(washing solution)*
- 15 mL conical tubes
- 6-well plates
- A centrifuge

Protocol:

1. **Cell preparation:**
    a. Thaw and recover LAD2/LADR cells following standard culture guidelines.
    b. Count cells once they have recovered; use 1-10 × 10^6 cells per condition; the example below uses 2 × 10^6cells/ condition.
    c. Removal of hSCF:
        i. Collect > 3×10^6 cells per condition in 15 mL tubes.
        ii. Centrifuge at 1500 rpm for 5 min and discard supernatant.
        iii. Resuspend in 2 mL and wash pellet 3 times with hSCF-free medium.
        iv. After final wash, resuspend in 1 mL hSCF-free medium and count cells.
2. **Seeding:**
    a. Seed 1×10^6 cells per well in 1 mL hSCF-free medium in 6-well plate:
        i. Well 1: **"Activated" (IgE treatment)**
        ii. Well 2: **"Unactivated" (control)**
    b. Incubate 4 hours at 37 °C, 5% CO_2.
3. **IgE sensitization:**
    a. Add the IgE to the "Activated" well to reach a **final concentration of 2 µg/mL**. Leave the unactivated well in hSCF-free medium as a control.

b. Incubate overnight at 37°C, 5% CO_2.

Note: If needed, pre-dilute IgE *(Y70000)* in hSCF-free medium before addition.

4. **IgE crosslinking:**
    a. Prepare washing solution: hSCF-free medium + 0.5% BSA.
    b. Wash activated cells 3 times with washing solution:
        i. Centrifuge at 1500 rpm for 5 min each time.
        ii. Resuspend pellet in 2 mL wash solution each wash.
    c. Resuspend the washed:
        i. Activated cells in **1 mL hSCF-free medium** containing **anti-human IgE** (Y79000) **at a final concentration of 1 µg/mL** in 6-well plate.
    d. Incubate 1 hour at 37°C, 5% CO_2.

Note: If needed, pre-dilute anti-IgE *(Y79000)* in hSCF-free medium before addition.

Please be advised that the washing step here is very critical. Complete wash away of the un-bound human IgE is important to get the correct testing result.

5. **Proceed to Mast Granulation Assay** *(G7800)*
    a. After the 1-hour IgE crosslinking step, activated and control cells are ready for downstream degranulation assay.

Tips:

- Ensure complete removal of hSCF before IgE sensitization to avoid baseline activation.
- Include unactivated controls to monitor background degranulation