De-granulation (β-hexosaminidase) Assay Protocol

Cat. T8157

Human Mast Cell Line (LAD2)

The Human Mast Cell Line (LAD2) may lose functionality after being passaged continuously for several months (approximately after 6-12 months). Therefore, functionality testing is recommended every 2 months.

Reagents Required:

HEPES
NaCl
KCl
$Na_2HPO_4 \cdot 7H_2O$
Glucose
PNAG (Sigma-Aldrich: N9376)
$CaCl_2 \cdot 2H_2O$
$MgSO_4 \cdot 7H_2O$
Citric acid
Glycine
Bovine Serum Albumin (BSA)

A. The following buffers are required:

1. ***HEPES buffer (1L)***

Component	Amount (g)	Final Concentration
HEPES	2.38	10 mM
NaCl	8.00	137 mM
KCl	0.2	2.7 mM
$Na_2HPO_4 \cdot 7H_2O$	0.103	0.4 mM
Glucose	1.008	5.6 mM

Add H_2O to 800 mL, adjust pH to 7.4 with NaOH (5M) and add:

$CaCl_2 \cdot 2H_2O$	0.265	1.8 mM
$MgSO_4 \cdot 7H_2O$	0.32	1.3 mM

Sterile filter and store at 4°C.

2. ***Citrate buffer (0.04 M, 200 mL)***

Component	Amount (g)	Final Concentration
Citric acid	1.6808	0.04 M
$Na_2HPO_4 \cdot 7H_2O$	1.0723	0.02 M

Add H_2O to 200 mL, adjust pH to 4.5 with NaOH (5M), sterile filter and store at 4°C.

3. ***Glycine (0.4 M, 500 mL)***

Component	Amount (g)	Final Concentration
Glycine	15.1	0.4M

Add H_2O to 500 mL, adjust pH to 10.7 with NaOH (5M), sterile filter and store at room temperature (RT).

4. ***HEPES + BSA (0.04%, 100 mL)***

Combine 100 mL HEPES buffer with 0.04 g BSA, sterile filter and store at 4°C.

B. Standard Procedure for β-hexosaminidase degranulation assay:

Day 1

5. Seed human mast cells at a density of 5-10x10^3 cells/well in 96 well plates. Prepare culture in cytokine depleted medium (PriGrow X Series Medium (TM8157) + 200mM L-Glutamine + 1% Penicillin/Streptomycin Solution) and add 100 ng/mL of fresh IgE[1]. Incubate over night.[2]

Day 2

6. Warm freshly prepared HEPES + 0.04% BSA buffer to 37°C.

7. Wash cells in HEPES + 0.04% BSA by centrifuging at 1000 rpm for 5 minutes. Repeat step 3 for a total of 3 times.

[1] IgE should be spun down, if possible at 100,000xg for 30 minutes or in a microfuge at maximal speed for 30 minutes at 4°C, to eliminate aggregates. Biotinylated human IgE is recommended as it crosslinks with Streptavidin to induce activation of the IgE receptor. If Biotinylated IgE is not available, normal human IgE can be used, and instead of streptavidin, anti-IgE can be used to stimulate the receptor.

[2] Cells are normally sensitized in one flask overnight to minimize variation in cell number between wells.

8. Re-suspend cells so that 90 µL equals the cell number needed for each well (total volume is 100 µL). Aliquot cells into a flat-bottom 96 well plate and incubate for 10 minutes at 37°C

9. Prepare antigen and human stem cell factor (hSCF) solutions in HEPES + 0.04% BSA. Make 10x dilutions.

   Example Antigen titration:

Final	10x Dilution
100 ng/mL	1000 ng/mL
10 ng/mL	100 ng/mL

   Add 10 µL buffer, antigen[3], SCF[4] or antigen + SCF and incubate for 30 minutes at 37°C.

10. Meanwhile, prepare 3.5 mg/mL PNAG solution in *citrate buffer* – sonicate to dissolve. Sufficient PNAG must be made for both supernatant and cell lysate (200 µL/sample). Aliquot 100 µL/well (two 96-well flat bottom plates are required; one for supernatant and one for lysate).

11. After incubation, spin plate at 1500 rpm for 3 minutes at 4°C.

12. Remove 50 µL of supernatant, add to 100µL PNAG, and incubate for 90 minutes at 37°C.

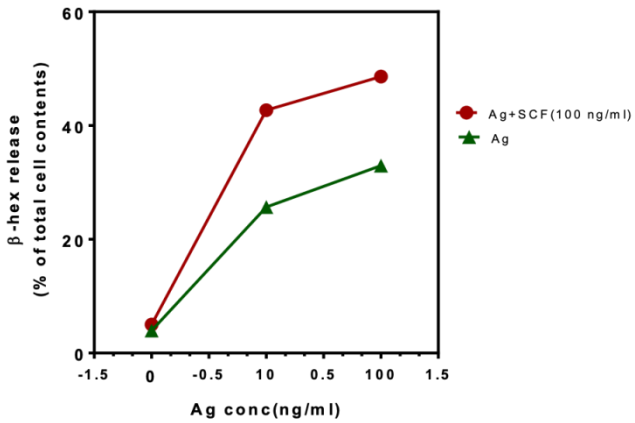
13. Lyse cells by adding 150 µL of 0.1% Triton X-100 (diluted in water) – pipette up/down 5 times, and transfer 50 µL of the lysis solution to the second PNAG plate and incubate for 90 minutes at 37 °C.

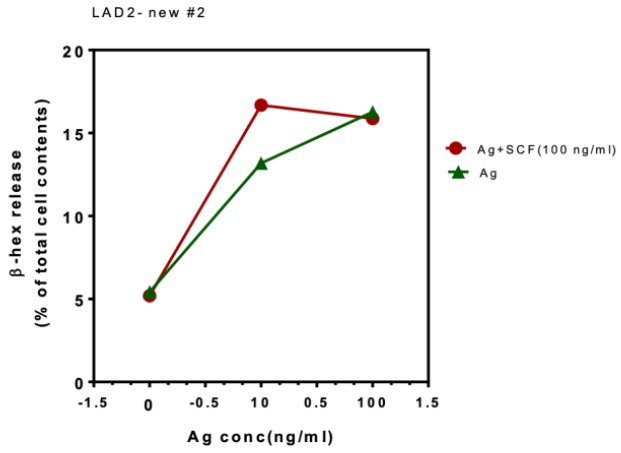
14. Stop reaction with 50 µL 0.4 M *Glycine solution* (color changes to yellow!) and read absorbance at 405 nm with a reference filter of 620 nm.

[3] The antigen is Streptavidin if biotinylated-IgE is used. Alternatively, use anti-IgE antibodies to crosslink IgE if normal human IgE is used for cell sensitization.

[4] SCF is the ligand for the c-KIT receptor which synergizes with the signaling of the IgE receptor. Normally stimulation with SCF causes an enhanced response to IgE/antigen challenge.

Example Results





Optimal conditions for beta-hex release should be determined by each laboratory.
100 ng/mL biotinylated human myeloma IgE has been previously used to sensitize LAD2/LADR cells; total release triggered by 100 ng/mL of streptavidin should range between 20-40%. Background (control) release is usually in the range of 2% to 5%.

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This protocol has been provided by the depositor in association with the publication: S. Kirshenbaum, A., Yin, Y., Sundstrom, J. B., Bandara, G., & D. Metcalfe, D. (2019). Description and characterization of a novel human mast cell line for scientific study. *International Journal of Molecular Sciences*, *20*(22), 5520. **abm does not warrant the accuracy of such information; all protocols must be experimentally tested by the end-user.**

Updated on May 24, 2023