Min Sample, Max Insight™

PRIMARY HUMAN MAST CELL ASSAY

RELIABLE EVALUATION OF DRUG EFFICACY USING HUMAN MAST CELL DEGRANULATION TEST

ABOUT MAST CELL ASSAY

Mast cells are granular immune cells that are characterized by c-kit and FcεR1 expression and originate from bone marrow blood precursors. Mast cells are involved in:

- Allergy and anaphylaxis
- Wound healing and angiogenesis
- Immune tolerance
- Defense against pathogens
- Vascular permeability in brain tumors

Mast cell degranulation can be induced by: IgE-FcεR1 complex aggregation, stem cell factor-induced c-kit dimerization, etc. During degranulation, mast cells release a number of immunomodulatory products that are implicated in both protective immune responses and clinically relevant disorders such as anaphylaxis.

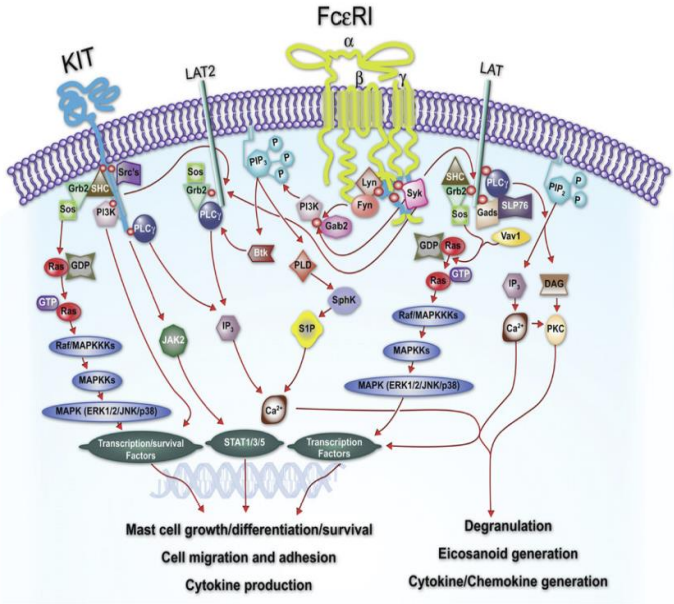
OUR ASSAYS WIDELY USED TO SYUDY BIOLOGY OF DISEASES

- ✓ Allergy
- ✓ Anaphylaxis
- ✓ Asthma
- ✓ IgE-mediated hypersensitivity reactions
- ✓ Mastocytosis
- ✓ Anti-helminth immunity
- ✓ Mast cell activation syndromes
- ✓ Cancer

At PicoImmune, we offer assays using human primary mature mast cells to determine the ability of a prospective therapeutic to modulate mast cell degranulation.

CHALLENGES OF MAST CELL RESEARCH

- ➢ Primary human mast cells are rare in peripheral blood and tissue isolation protocols yield low cell quantities, viability and purity.
- ➢ Differentiation of blood stem/precursor cells to mature mast cells is time-consuming and culture reagents are costly.
- ➢ Both human (HMC-1, LAD2, LUVA) and rodent (P815, RBL-1, FMA-3) mast cell lines exhibit aberrant phenotypes, including lack/loss of functional receptors such as FcεR1, impaired cytokine production, long doubling time or insufficient cytosolic granules.



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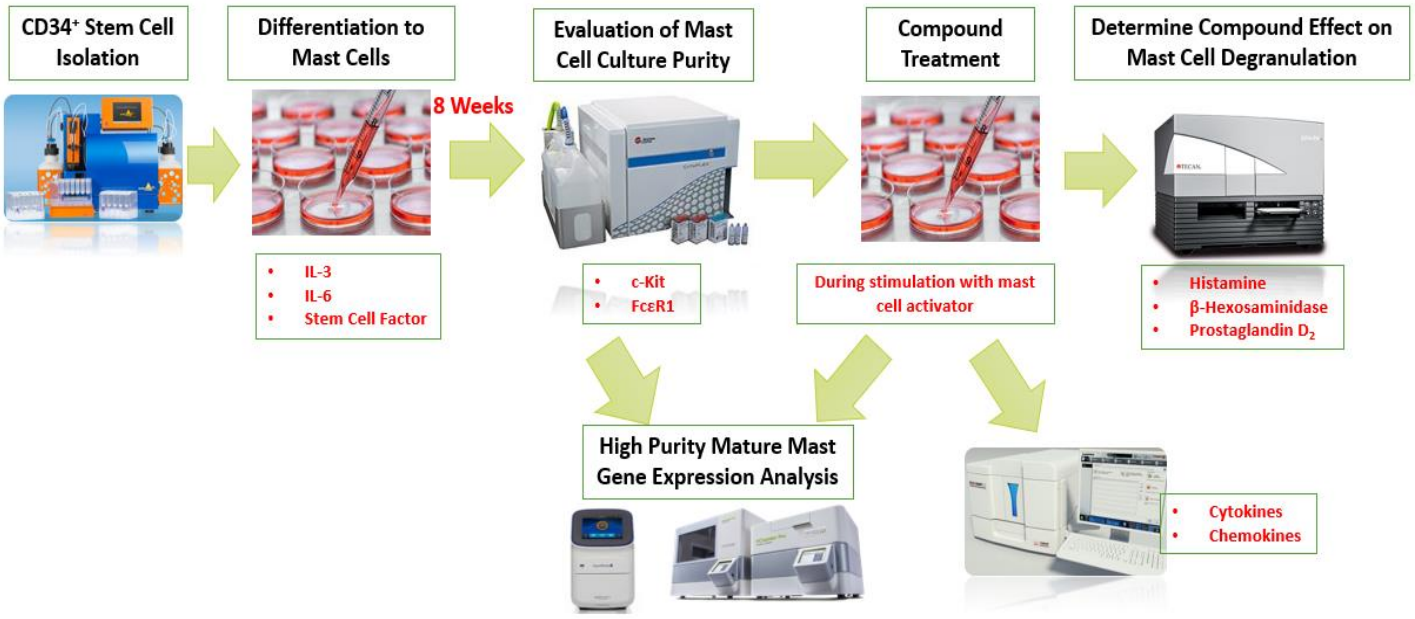
OUR SERVICE FEATURES

- ❖ **CD34+ blood precursor-derived mast cells**: Mimic mature human mast cells; express functional c-kit and FcεR1; have a well condensed non-lobate nucleus and abundant cytosolic granules
- ❖ **Variety of readouts**: histamine/ tryptase/ β-hexosaminidase/cytokines/ PGD2/gene expression
- ❖ **Degranulation Induction:** IgE-dependent and -independent (Compound 48/80, cortistatin-14, substance P)
- ❖ **High throughput**: 96/384-well format, duplicate or triplicate; multiple donors can be tested concurrently; highly multiplex analysis at transcriptome/secretome/proteome levels
- ❖ **Robust and highly reproducible:** More predictive results than with cell lines or rodent cells
- ❖ **Well-validated reagents and protocol:** provide established differentiation reagents, mast cell agonists and antagonists
- ❖ **Turnaround time**: ~3 months
- ❖ **State-of-art platforms**: CytoFLEX Flow Cytometer; FlexMAP 3D (Luminex, 96 and 384-well format); Tecan Microplate Reader; NanoString nCounter
- ❖ **20+ years of experience of mast cell research**: Expert data analysis and interpretation, scientific and technical support

HOW OUR ASSAY WORK?

1. CD34+ precursor cell isolation from peripheral blood.
2. Differentiation of CD34+ precursor to mature mast cells.
3. Evaluation of mast cell culture purity:
   - c-kit expression
   - FcεR1 expression
4. Test compound treatment during stimulation
5. Readout quantification:
   - Cytokines production
   - Gene Expression
   - Degranulation products/markers



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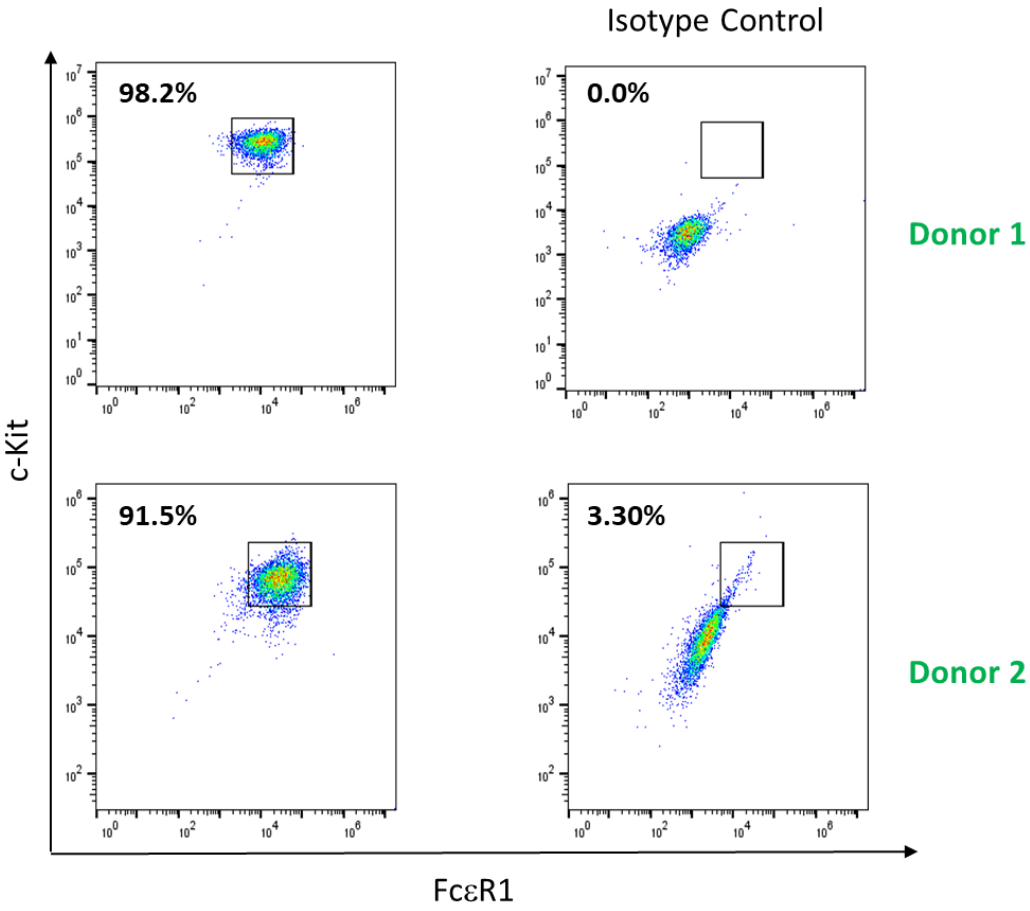
EXAMPLE DATA 1: MAST CELL PURITY

Primary CD34+ hematopoietic precursor cells isolated from 2 healthy donors were differentiated to mast cells *in vitro* by culturing cells in medium supplemented with recombinant stem cell factor (SCF), interleukin (IL)-6, and IL-3. Mast cell culture purity was evaluated after weeks of differentiation based on the expression of c-kit and FcεR1 with flow cytometry.



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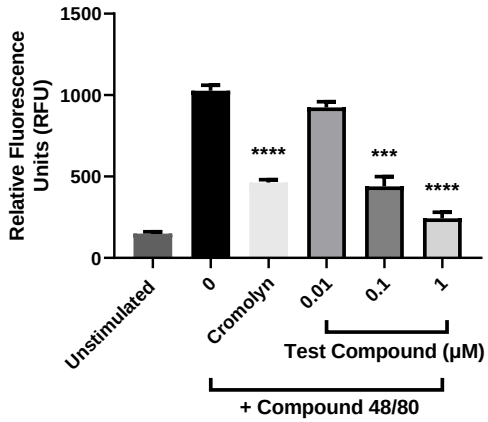
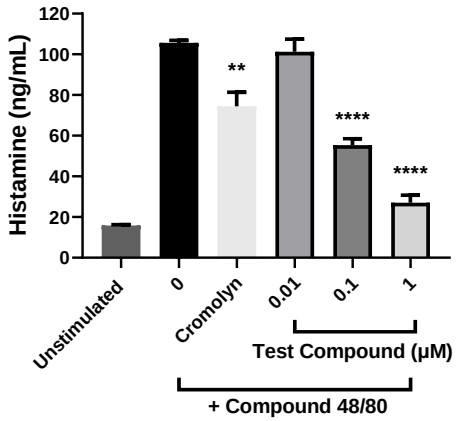
EXAMPLE DATA 2: COMPOUND 48/80 INDUCED MAST CELL DEGRADNULATION

Mature CD34+ hematopoietic precursor-derived mast cells were incubated with varying concentrations of test compound or cromolyn (mast cell stabilizer) during stimulation with compound 48/80 (mast cell activator). After treatment, β-hexosaminidase, histamine, prostaglandin D_2 and tumor necrosis factor (TNF)-α in the cell-free supernatants were quantified. Data shown are mean ± SEM (3 donors). Statistical analysis was performed using one-way ANOVA with pairwise comparisons made to cells that received no compound treatment. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

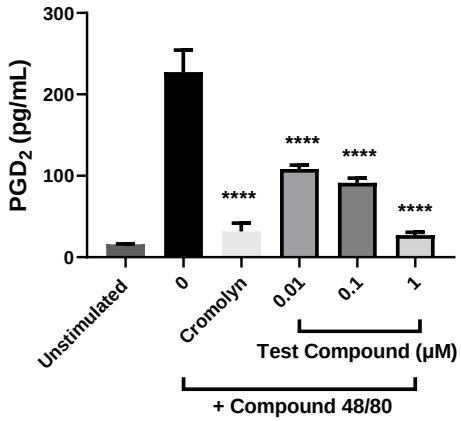
β-Hexosaminidase



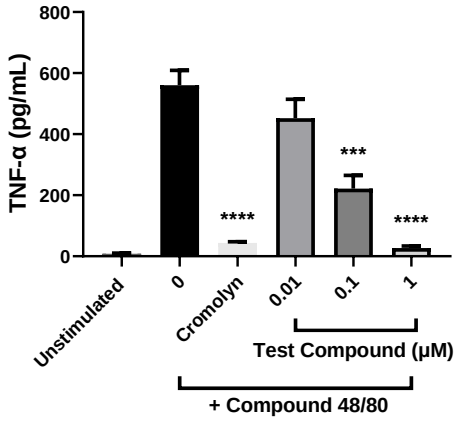
Histamine



Prostaglandin D_2



Tumor Necrosis Factor-α



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