PRIMARY HUMAN MAST CELL ASSAY

Accurate and precise evaluation of test compound effects on primary mast cell degranulation

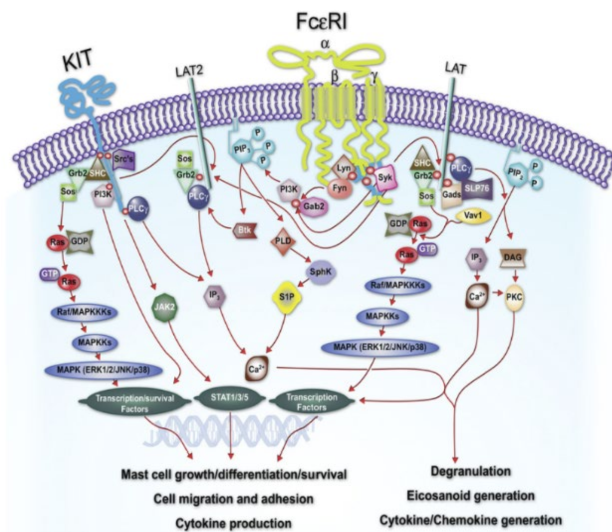
ABOUT MAST CELL ASSAY

Mast cells (MCs) play a central role in allergic inflammation and peripheral immune defense. Their activation can trigger severe, even life-threatening responses such as **asthma** and **anaphylaxis**. As a result, targeting mast cell survival, activating receptors, and the release of key mediators have become a priority in immunological drug development.

Axela offers advanced ***in vitro* mast cell assay platforms** designed to evaluate compound impact on mast cell function, mediator release, and activation pathways. Our robust, reproducible systems help accelerate early-stage discovery and derisk preclinical decisions.

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.



OUR ASSAY CAPACBILITIES

- **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
- **Turnaround time**: ~3 months
- **Variety of readouts**: histamine: histamine/ tryptase/ β-

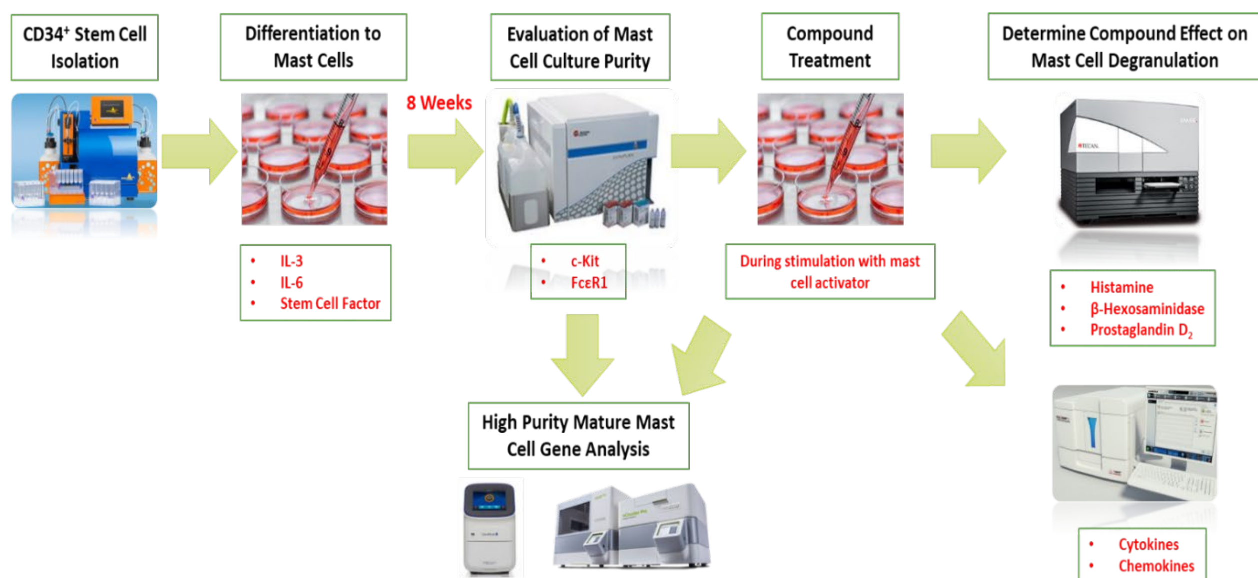
hexosaminidase/cytokines/PGD2/gene expression

- **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
- **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, interpretation, scientific and technical support

HOW DOES OUR ASSAY WORK?

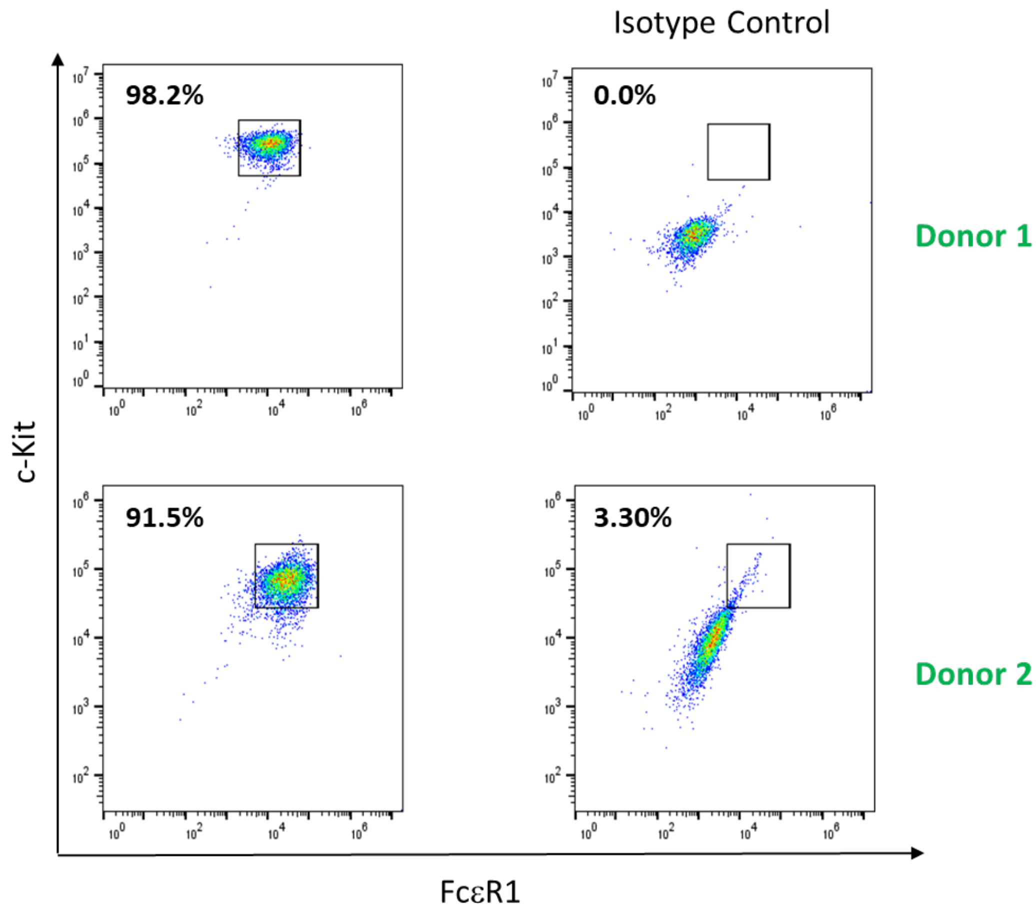
1. CD34+ precursor cell isolation from peripheral blood.
2. Differentiation of CD34+ precursor to mature mast cells.
3. Evaluation of mast cell culture purity:
   - c-kit expression
   - FcεR1 expression
4. Test compound treatment during stimulation
5. Readout quantification:
   - Cytokines production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

THE WORKFLOW OF OUR ASSAY



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.



EXAMPLE DATA 2: COMPOUND 48/80-INDUCED MAST CELL DEGRANULATION

Mature CD34+ hematopoietic stem cell-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.