



Eosinophil Degranulation and Activation Assay Services

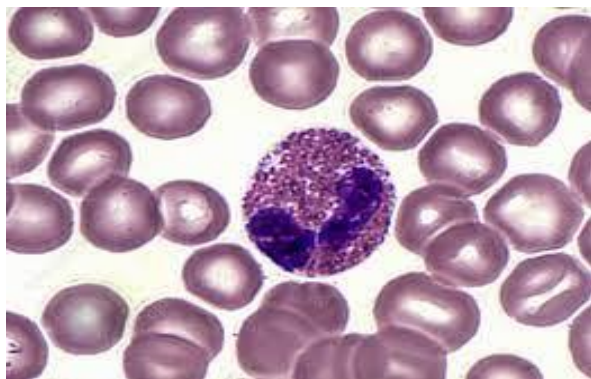
Accurate evaluation of eosinophil function and activity

EOSINOPHIL® ASSAYS

Eosinophils are granular myeloid immune cells that are primarily found in tissues with only a few present in peripheral blood. They are well known for their protective response against helminths. Most of their cytotoxic functions are due to the major basic and cationic proteins contained within their abundant cytosolic granules. Despite their prominent role in host defense, eosinophils contribute to the pathogenesis of several diseases including allergies, chronic obstructive pulmonary disease and colitis, where they cause significant tissue destruction.

Given the contribution of eosinophils to host defense and disease, eosinophils represent an ideal target for developing novel therapies.

At Axela, our scientists routinely screen compounds for their ability to modulate eosinophil function.



HOW OUR ASSAY WORKS

A typical workflow includes:

- ✓ Eosinophil sourcing:
 - Primary cells: Isolation from peripheral whole blood from multiple donors and confirmation of purity (CD66b and CD16 expression)
 - Cell Line: Onsite inventory of EoL-1
- ✓ Test compound treatment
- ✓ Stimulation of pre-treated eosinophils (necessity varies with assay)
 - Phorbol 12-myristate 13-acetate (PMA)
 - A-23187
 - Cytokines (IL-4, IL-13, Eotaxin, etc)
 - Platelet-Activating Factor (PAF)
- ✓ Analyte quantitation: Degranulated mediator, CD63 expression, EETosis (Eosinophil Extracellular Trap formation), Chemotaxis

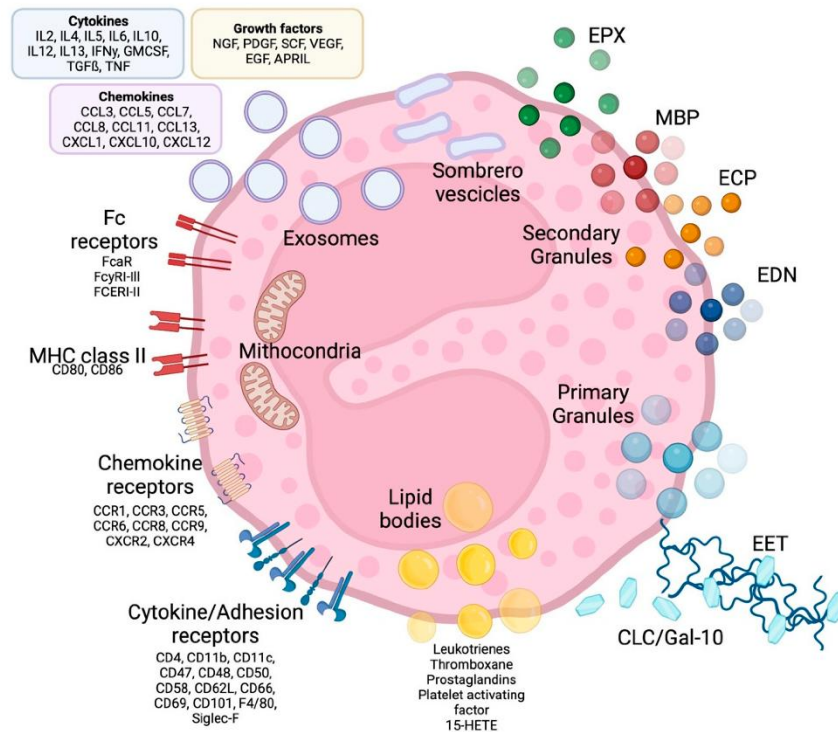
OUR ASSAY BENEFITS

- ✓ Superior sensitivity: Measure eosinophil activation at the single cell level.
- ✓ High throughput: 96 - 384 well assay formats
- ✓ Fully validated and quality-controlled: Assays are optimized for accurate and reproducible data.
- ✓ Positive and negative controls are also included.
- ✓ Flexibility: Easily automated for use in small to large scale screening compound screening
- ✓ Simultaneous analyte detection: Monitor degranulation and activation simultaneously.



- ✓ State-of-art platforms: Cytoflex S flow cytometer (4 lasers, 13 colors), Tecan Spark multimode microplate reader, FlexMAP3D and Luminex 200 systems.
- ✓ Multiple endpoints to clearly understand the immunomodulatory profile of test agents: Extracellular trap formation, degranulation (eosinophil peroxidase, CD63 expression), cytokine production (IL-3, IL-9, IL-13, IL-5, etc)
- ✓ Timely data delivery: 1-4 weeks or sooner, upon receiving test samples
- ✓ 50+ years of accumulated experience in immunology: Extensive expert data analysis and interpretation, high quality scientific and technical support

Eosinophils Are Involved in Homeostatic and Inflammatory Responses



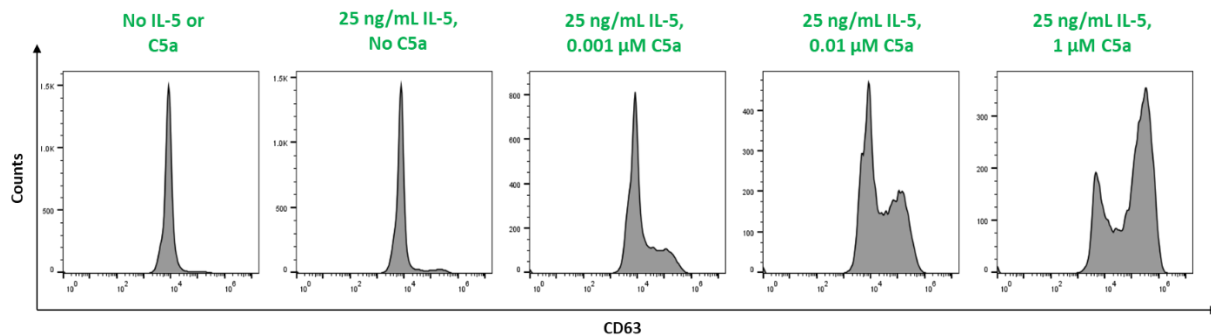
CLC/Gal-10: Charcot-Leyden/galectin-10 protein;
 ECP: eosinophil cationic protein;
 MBP: major basic proteins;
 EPX: eosinophil peroxidase;
 EDN: eosinophil-derived neurotoxin;
 MHC class II: Major histocompatibility complex-II;
 EET: eosinophilic extracellular traps



OUR DATA EXAMPLES

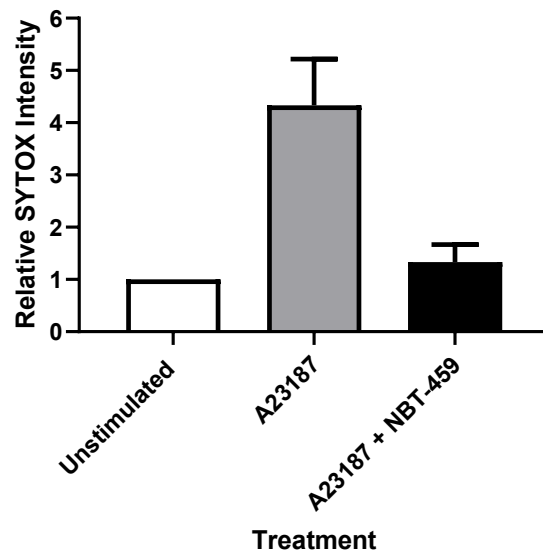
Example 1. Degranulation of cytokine primed-human eosinophils.

Human primary eosinophils were primed with IL-5 and then stimulated with complement factor 5a (C5a). CD63 cell surface expression, a degranulation marker, was then monitored using flow cytometry. Histograms are representative data from triplicate samples.



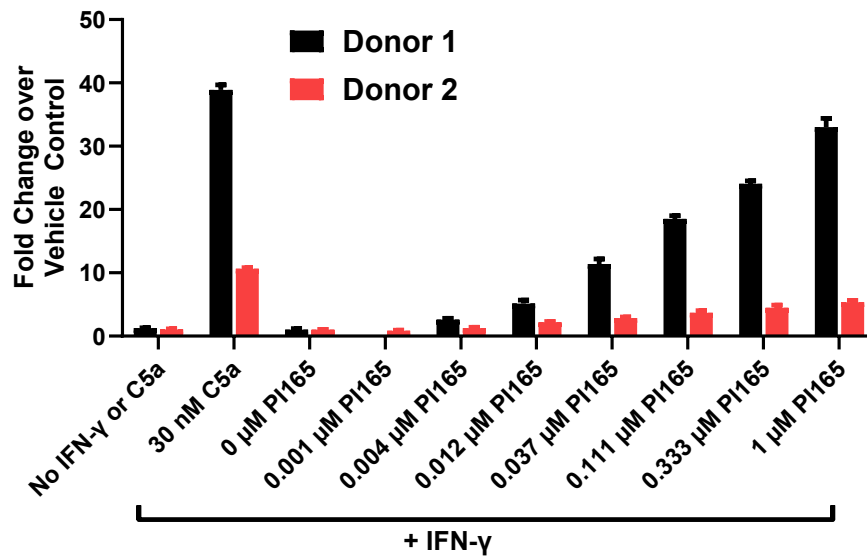
Example 2. The Effect of NBT-459 on Eosinophil Extracellular Trap Formation.

Eosinophils, from healthy donors, were treated with or without test compounds during A23187 stimulation. Extracellular trap formation was monitored by using a fluorescent, membrane-impermeable DNA-intercalating dye. Fluorescence intensity is expressed relative to unstimulated control. The results are mean \pm SD of technical triplicates.



Example 3. The Effect of PI-165 on Human Eosinophil Degranulation.

Human eosinophils from 2 donors were stimulated with IFN- γ and complement factor 5a (C5a), positive control, or varying concentrations the test compound PI-165. Released eosinophil granule protein, eosinophil peroxidase (EPX), in the cell supernatant was monitored using a colorimetric assay. Data are represented as a fold of change over vehicle control (25 ng/mL IFN- γ , 0 μ M test compound). Values are mean \pm SD of technical triplicates.



Example 4. The Effect of Test Compounds on IL-4-dependent CCL17 Production.

Eosinophils, from healthy donors, were treated with varying concentrations of test compounds during IL-4 stimulation. After treatment, the cell-free supernatant was collected, and quantity of cytokines was determined using Luminex assay. Data shown for CCL17 (TARC) are mean \pm SD of technical triplicates.

