LADR and LAD2 Functional Validation

Product: LADR Mast Cells (Cat. No. T8156) & LAD2 Mast Cells (Cat. No. T8157)
Assay: Mast Cell Granulation Assay (Cat. No. G7800)

To evaluate the functional activity of mast cells, LADR and LAD2 cells were subjected to the Mast Granulation Assay following IgE sensitization with human IgE (Cat. No. Y70000) and IgE crosslinking (Cat. No. Y79000). Degranulation was quantified by measuring p-nitroaniline (pNA)* release in the supernatant (released granules) and lysate (retained granules).

**Note: p-Nitroaniline (pNA) is not histamine and is not a naturally occurring marker of histamine release. Instead, pNA is a chromogenic substrate used in laboratory assays to quantify tryptase activity, an enzyme co-released with histamine during mast cell degranulation. The amount of pNA generated is therefore an indirect but reliable indicator of mast cell activation.*

Results Summary

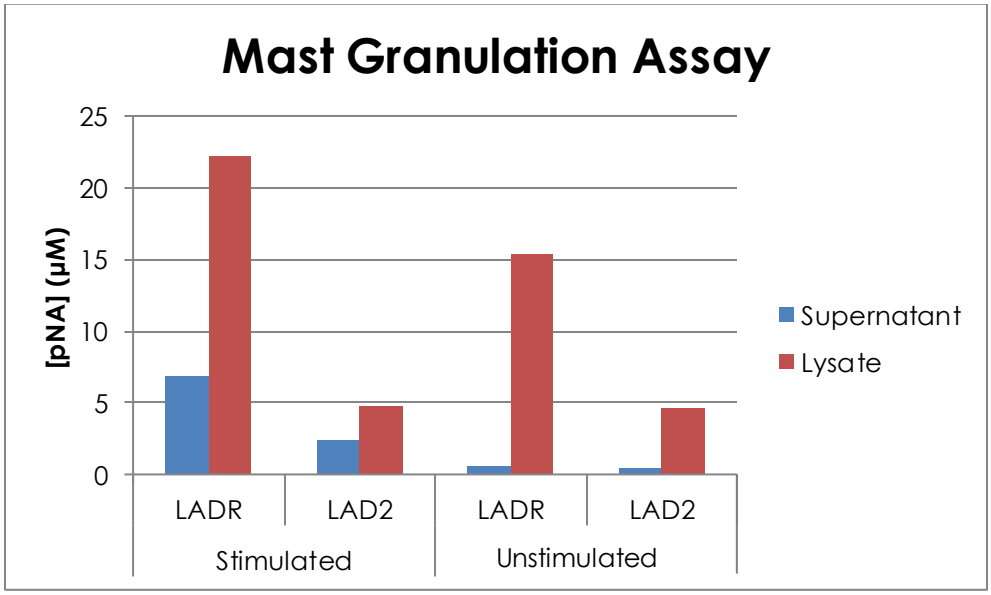

Figure 1: IgE-mediated degranulation of LADR and LAD2 mast cells. *Only activated cells trigger a significant rise in pNA release, demonstrating that both cell lines retain robust, stimulus-dependent mast cell functionality.*

Data Analysis

Upon stimulation, both LADR and LAD2 cells demonstrated stimulus-dependent degranulation, with LADR showing a stronger overall response. Both cell lines maintain low baseline degranulation in the absence of stimulation.

LADR Observations	
Stimulated	• High pNA levels in the lysate, indicating a substantial store of granules. • Significant increase in supernatant pNA, confirming robust granule release following activation.
Unstimulated	• Elevated lysate pNA levels consistent with stored granules. • Minimal pNA release into the supernatant, confirming tight regulation of granulation without a trigger.

LAD2 Observations	
Stimulated	• Moderate lysate pNA levels with measurable release into the supernatant. • Activation response is present, though lower in magnitude compared to LADR.
Unstimulated	• Lower lysate pNA levels compared to LADR, consistent with known cell-line differences. • Supernatant pNA nearly absent, indicating minimal spontaneous release.

Conclusion

Both LADR and LAD2 maintain stable, quiescent baseline behavior, with little to no spontaneous degranulation. Both cell lines respond to IgE crosslinking with measurable degranulation, with LADR exhibiting a stronger activation profile.