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LUVA- human mast cell line

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Origin: Mast cells were grown from $CD34^+$-enriched mononuclear cells derived from the peripheral blood of a donor with aspirin exacerbated respiratory disease according to the methodology of Kirshenbaum et al. (*Blood 1999; 94,7: 2333*). The patient had no clinical symptoms consistent with mastocytosis or leukemia and has not developed any as of this time (>2 years after blood donation). Cells were cultured for 1 week with SCF (100 ng/ml), IL-6 (50 ng/ml), and IL-3 (10 ng/ml) and subsequently hemidepleted every week and given fresh media, IL-6, and SCF. After 8 weeks, the cells continued to increase in number at an atypical rate, and their dependency on exogenous cytokines for survival and proliferation was lost. The cells continue to grow independent of stem-cell factor. Sequencing of the c-kit gene revealed no mutations and the c-kit signaling pathway was functional, but not altered in terms of activity when compared to other mast cell lines.

Culture medium: STEM PRO-34 SFM (Gibco #10640 500 ml bottle)
Supplements: per 500 ml bottle- STEM PRO-34 nutrient supplement (Gibco #10641-025); Pen Strep 5ml (Gibco #15140); L-glutamine-200 mM 5 ml (Gibco # 25030-081); and Primocin 1 ml (50 mg)
Passage/Feeding Frequency: The cells grow best when the flask is left upright. The cells will need to be split or have the media changed every 2-3 days.
Split Ratio: Cells can be split 1:2-1:5 depending on how often cells are needed for experiments.
Freezing Medium: Long-term storage of cells is performed according to the protocol of Kirshenbaum et al. (Leukemia Research 2003; 27:677). Cells are suspended in non-DMSO, non-FBS containing pZerve cryopreservative (Protide Pharmaceuticals; http://www.protidepharma.com/products_celox.php) ($5x10^6$ cells per l ml), incubated for 30 min at 22 °C, transferred to -20 °C for 1 hr, then -70 °C for 1 hr and placed in liquid N_2. An alternative method is to use the same number of cells, but use freezing medium instead (92 ml STEM-PRO and 8 ml DMSO).
Freeze/thaw Protocol: Warm the vial at 37 °C until the solid just turns to liquid. Wash vial with 70% ethanol and transfer contents to 15 ml tube and add 5 ml culture medium. Mix the cells and centrifuge to pellet washed cells. Resuspend in 5 ml culture medium and place in culture flask upright in 37 °C incubator with 95% air and 5% CO_2.

References:

1. Kirshenbaum AS, Goff JP, Semere T, Foster B, Scott, LM and Metcalfe DD Demonstration that human mast cells arise from a progenitor cell population that is $CD34^+$, *c-kit*$^+$, and expresses aminopeptidase N (CD13) Blood 1999; 94,7: 2333-2342
2. Kirshenbaum AS, Akin C, Wu Y, Rottem M, Goff JP, Beaven MA, Rao K, Metcalfe DD Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of FcεRI or FcγRIα Leukemia Research 2003; 27: 677-682
3. Laidlaw, T.M., Steinke, J.W., Tiñana, A.M., Xing, W.,Lam, B.K., Paruchuri, S., Boyce, J.A., and Borish, L. Characterization of a novel human mast cell line that responds to stem cell factor and expresses functional FcεRIα Journal of Allergy and Clinical Immunology (2011) in press