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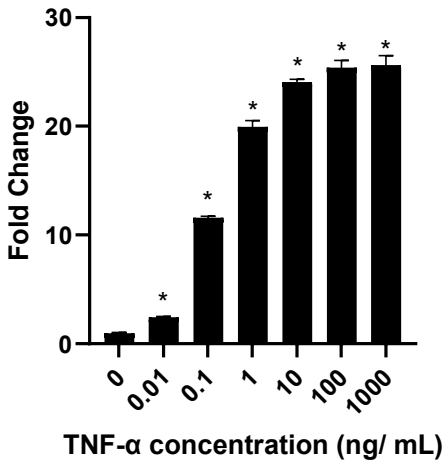
Technical Data Sheet: HMC3 NFκB-Luc2

ATCC® Number	CRL-3304-NFκB-LUC2™
Organism	*Homo sapiens*
Tissue/Disease Source	Microglial Cell; Brain (Normal)
Product Description	HMC3 (ATCC® CRL-3304) is a microglial cell line isolated from a patient brain that was deposited by KH Krause (University of Geneva, Switzerland). HMC3 NFκB-LUC2 luciferase reporter cell line was derived from parental line HMC3 by stably expressing firefly luciferase gene (luc2) under control of the nuclear factor kappa B (NFκB) promoter. This cell line was established through lentiviral transduction and single cell cloning. The cells, upon stimulation, express high levels of enzymatically active luciferase protein, which can be detected via *in vitro* bioluminescence assays. This reporter cell line is useful for monitoring the activity of tumor necrosis factor alpha (TNF-α)-induced signal transduction pathways.
Application	Enabling sensitive and quantitative assessment of signal transduction makes this reporter cell line ideal for in vitro bioluminescence assays to study immune response in cell lines with high expression of PD-L1 and SIRPα, development of new drugs, and safety evaluation of new chemicals and drugs.

In vitro expression of luciferase by TNF-α and T cell-conditioned media

A **Luminescence signal from HMC3 NFκB-Luc2 upon TNF-α stimulation (Fold change)**



B **Luminescence intensity of HMC3 NFκB-Luc2 upon T cell-conditioned media stimulation**

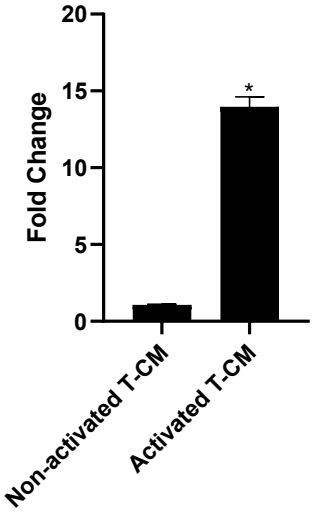


Figure 1. In vitro expression of luciferase by TNF-α and T cell-conditioned media. Luciferase expression from HMC3 NFκB-Luc2 cells upon signaling activation by (A) TNF-α stimulation (0.01 – 1,000 ng/ mL), (B) conditioned-media stimulation from checkpoint matched non-activated and activated primary CD4+ T cells. N=3 in all experiments. *, $P < 0.05$.

In vitro expression of luciferase in co-culture assay

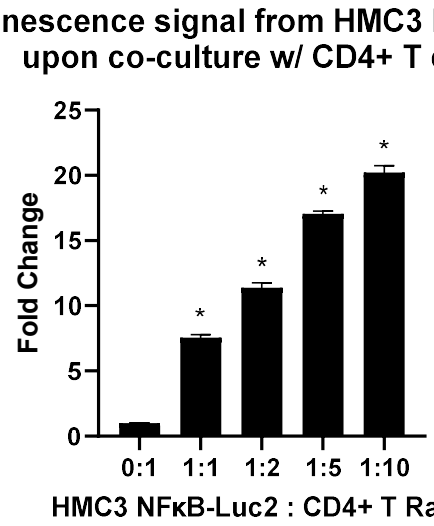


Figure 2. In vitro expression of luciferase in co-culture assay. HMC3 NFκB-Luc2 cells were co-cultured with varying ratios of primary CD4+ T cells for 24 hours. N=3 in all experiments. *, P < 0.05.

Biomarker expression

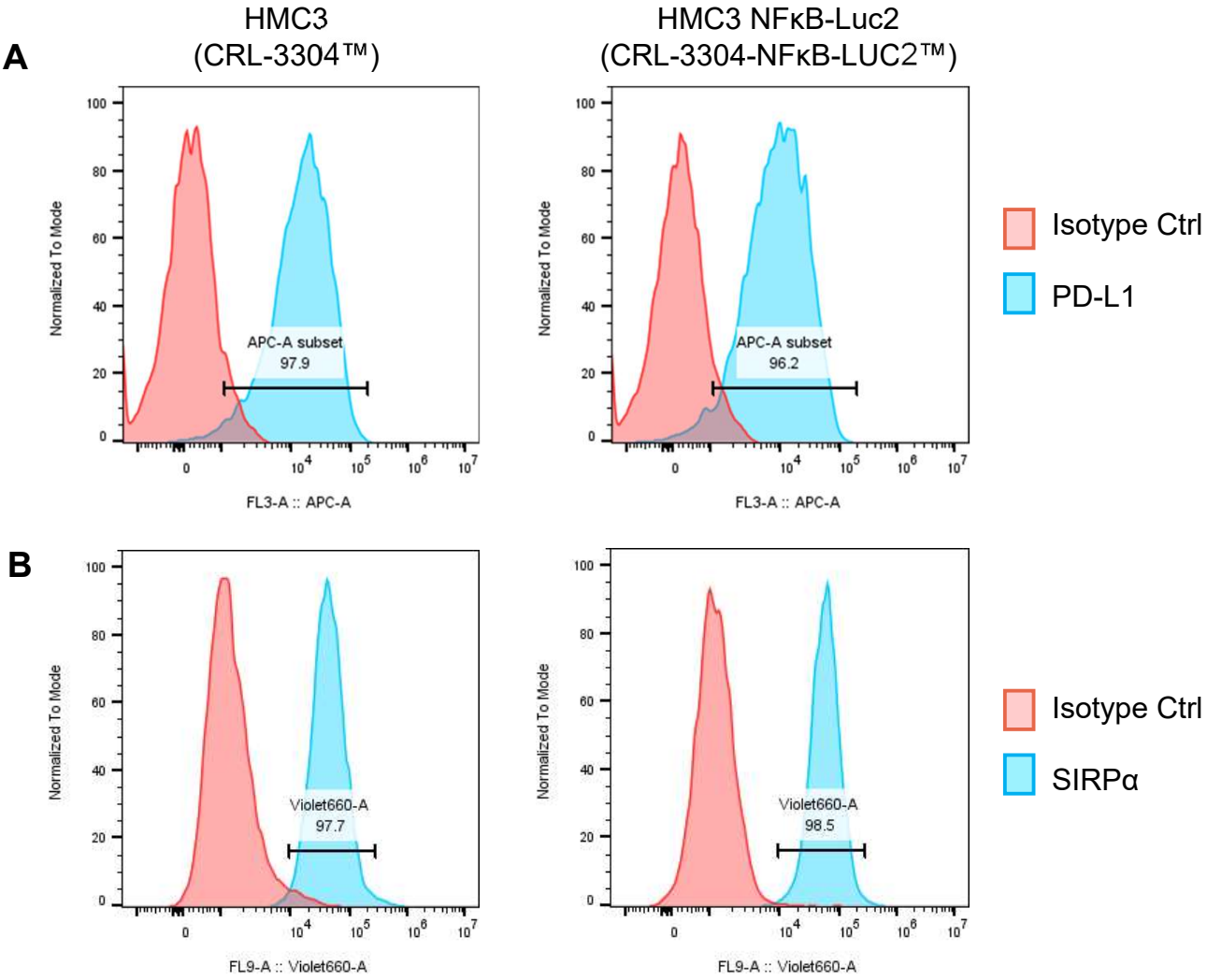
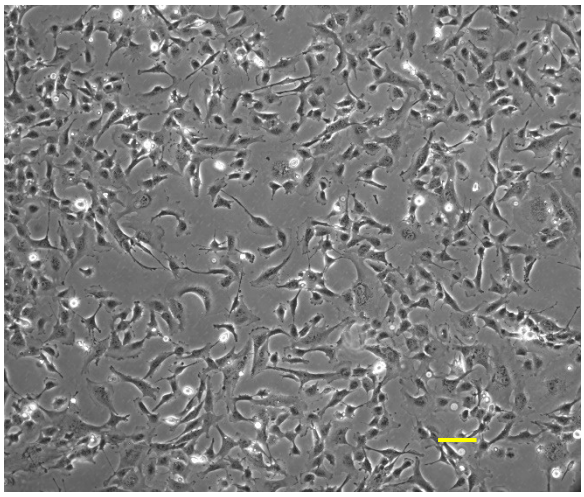


Figure 3. Biomarker expression of HMC3 parental and HMC3 NFκB-Luc2. The expression of (A) PD-L1 and (B) SIRPα on the cell surface of HMC3 parental and NFκB-Luc2 cell lines was evaluated by flow cytometry.

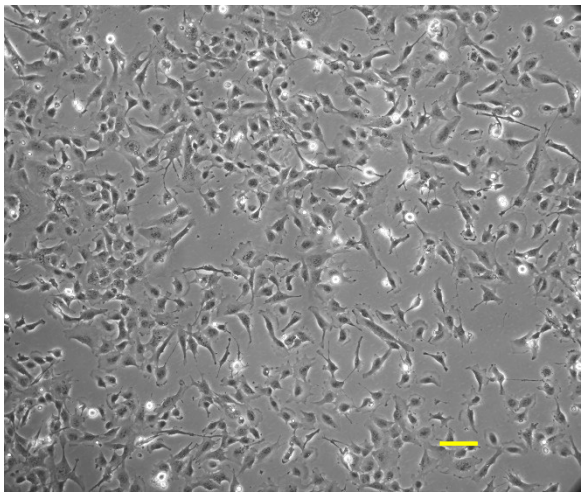
Cell Morphology

HMC3
(CRL-3304™)



Doubling time = 31.9 hours

HMC3 NFκB-Luc2
(CRL-3304 NFκB-LUC2™)



Doubling time = 38.9 hours

Figure 4: Cell morphology of HMC3 parental and HMC3 NFκB-Luc2. Cells were maintained in ATCC recommended culture conditions. Cell morphology was observed under microscopy and images were captured by digital camera. Scale bars represent 100 µm.