

Technical Data Sheet: U-937-NFkB-Luc2

ATCC® Number	CRL-1593.2-NFκB-LUC2™
Organism	Homo sapiens
Tissue/Disease Source	Pleural effusion/ Histiocytic lymphoma
Product Description	U-937 (ATCC® CRL-1593.2 [™]) is a cell line exhibiting monocyte morphology that was derived in 1974 from malignant cells obtained from the pleural effusion of a 37-year-old, white, male patient with histiocytic lymphoma. U-937-NFκB-LUC2 luciferase reporter cell line was derived from parental line U-937 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 <i>in vitro</i> 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 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 U-937 NFkB-Luc2 B Luminescence signal from U-937 NFkB-Luc2 upon TNF-α stimulation

1000-800 Fold Change 600 400 200-1 10,00,00 000,00

TNF-α concentration (ng/ mL)

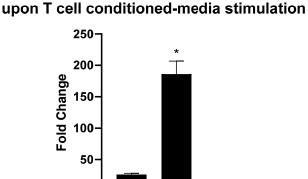


Figure 1. In vitro expression of luciferase by TNF-α and T cell-conditioned media. Luciferase expression from U-937-NFκB-Luc2 cells upon signaling activation by (A) TNF-α stimulation (0.01 – 1,000 ng/ mL), (B) conditioned-media stimulation from checkpoint matched non-activated and activated primary CD8+ T cells. N=3 in all experiments. *, P < 0.05.

In vitro expression of luciferase in co-culture assay

Luminescence signal from U-937-NFkB-Luc2 upon co-culture w/ CD4+ T cells

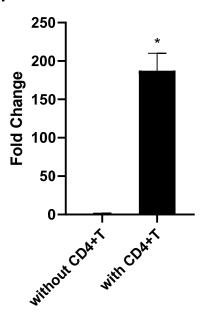


Figure 2. In vitro expression of luciferase in co-culture assay. U-937-NF κ B-Luc2 cells were co-cultured with 1:1 (E:T) ratio of primary CD4+ T cells for 6 hours. N=3 in all experiments. *, P < 0.05.

Biomarker expression

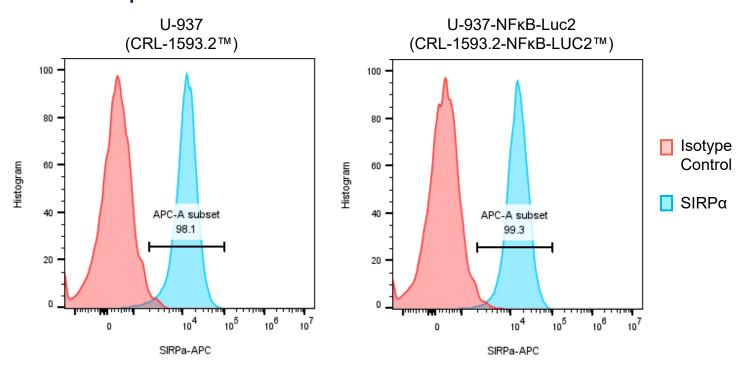
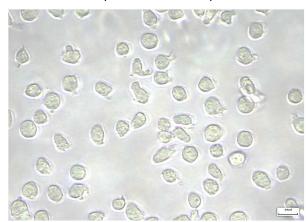


Figure 3. Biomarker expression of U-937 parental and U937-NFκB-Luc2. The expression of SIRP α on the cell surface of U-937 parental and NFκB-Luc2 cell lines was evaluated by flow cytometry.

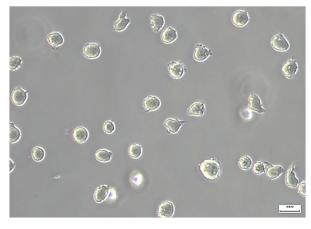
Cell Morphology

U-937 (CRL-1593.2™)



Doubling time = 19.7 hours

U-937-NFkB-Luc2 (CRL-1593.2-NFkB-LUC2™)



Doubling time = 19.2 hours

Figure 4: Cell morphology of U-937 parental and U-937-NFκB-Luc2. Cells were maintained in ATCC recommended culture conditions. Cell morphology was observed under microscopy and images were captured by digital camera.

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