

Technical Data Sheet: <u>KG-1 NFκB-Luc2</u>

ATCC [®] Number	CCL-246-NFκB-LUC2™
Organism	Homo sapiens
Tissue/Disease Source	Bone marrow/ Acute myelogenous leukemia
Product Description	KG-1 (ATCC [®] CCL-246 [™]) is a cell line made up of macrophages isolated from a bone marrow aspirate obtained from a white, 59-year-old male with erythroleukemia that evolved into acute myelogenous leukemia. KG-1 NFκB-LUC2 luciferase reporter cell line was derived from parental line KG-1 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 Siglec-10, 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 sinal from KG-1 NFkB-Luc2 upon TNF-α stimulation (Fold change)
 - $\frac{200}{150}$
- B Luminescence intensity of KG-1-NFkB-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 KG-1 NF κ B-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 KG-1 NFkB-Luc2 upon co-culture w/ CD4+ T cells

Figure 2. In vitro expression of luciferase in co-culture assay. KG-1 NFκB-Luc2 cells were cocultured 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 KG-1 parental and KG-1 NFkB-Luc2. The expression of Siglec-10 on the cell surface of KG-1 parental and NFkB-Luc2 cell lines was evaluated by flow cytometry.

Cell Morphology



Doubling time = 38.6 hours

Doubling time = 38.9 hours

Figure 4: Cell morphology of KG-1 parental and KG-1 NFkB-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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