Validation data for THP1-Blue™ KI-IP10 cells

https://www.invivogen.com/thp1-blue-ki-ip10

For research use only

Version 19A09-ED

THP1-Blue™ KI-IP10 cells were generated by knockin of the Lucia luciferase reporter gene in place of the complete open reading frame of IP10 (CXCL10) in THP1-Blue™ NF-κB cells. Thus, the Lucia luciferase reporter is under the control of the endogenous CXCL10 promoter, allowing the study of CXCL10 expression specifically (**Figure 1**). Additionally, THP1-Blue™ KI-IP10 cells can be differentiated into macrophages using Phorbol 12-myristate 13-acetate (PMA), and CXCL10 expression, a classical marker of pro-inflammatory M1 macrophages, can be measured (**Figure 2**). These cells also stably express the NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene in response to the activation of the NF-κB pathway (**Figure 3**). This can be used for studying the activation of NF-κB in combination with CXCL10 expression, which may aid in elucidating crosstalk that might occur between the two signaling pathways.

Evaluation of CXCL10 (IP10) induction (Lucia luciferase reporter)

The expression of Lucia luciferase in THP1-Blue™ KI-IP10 cells in response to various agonists including STING ligands (cyclic dinucleotides (CDNs)) and type I IFNs has been assessed. These cells respond strongly to CDNs including 2'3'-cGAMP and 2'3'-cGAM(PS)$_2$ (Rp,Sp), and to a lesser extent RNA, DNA, and type I IFNs (IFN-α/β) (**Figure 1**). No CXCL10 expression was detected in the cell line when stimulated with TNF-α. With a similar detection range to conventional methods such as ELISA and reverse-transcription quantitative-PCR (RT-qPCR), THP1-Blue™ KI-IP10 cells provide a more convenient approach to quantitatively measure endogenous CXCL10 expression.

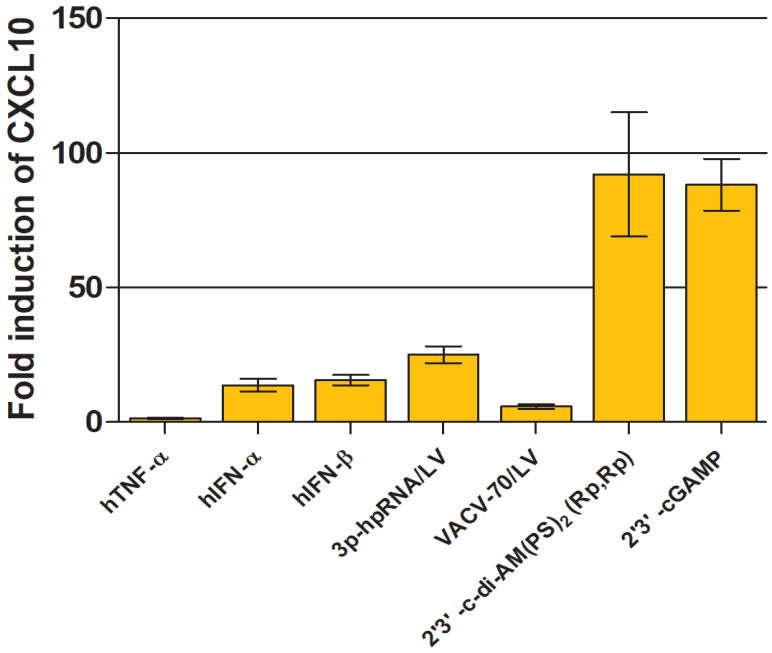

Figure 1: THP1-Blue™ KI-IP10 cells were stimulated with human (h)TNF-α (10 ng/ml), hIFN-α (1 x 10^4 U/ml), hIFN-β (1 x 10^4 U/ml), 3p-hpRNA/LyoVec™ (LV) (1 µg/ml), VacV-70/LyoVec™ (LV) (1 µg/ml), 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) (30 µg/ml), and 2'3'-cGAMP (30 µg/ml). After 24h, CXCL10 expression was determined by measuring the relative light units (RLUs) in a luminometer using QUANTI-Luc™, a Lucia luciferase detection reagent. Fold induction was calculated by taking into account the non-induced background for each ligand.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

CXCL10 is a marker of M1 macrophages

There are two major polarization states for macrophages, the classically activated type 1 (M1) and the alternatively activated type 2 (M2). CXCL10 is a typical marker of the pro-inflammatory response of M1 macrophages. The expression of Lucia luciferase in THP1-Blue™ KI-IP10 cells after differentiation into M0, M1, and M2 macrophages has been assessed. As expected, the expression of CXCL10 is exclusively seen in M1 macrophages (**Figure 2**). This expression can be completely blocked by using a JAK1/JAK2 inhibitor such as Ruxolitinib.

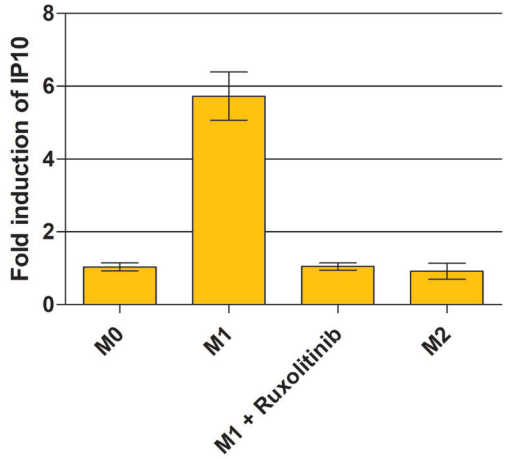

Figure 2: For differentiation into M0 macrophages THP1-Blue™ KI-IP10 cells were stimulated overnight with PMA (150 nM). Then for differentiation into M1 macrophages, the cells were exposed to IFN-γ (200 ng/ml) and LPS (10 pg/ml) with and without the JAK/STAT inhibitor Ruxolitinib (1 µM), and for differentiation into M2 macrophages the cells were exposed to IL-4 (20 ng/ml) and IL-13 (20 ng/ml). After 24h, CXCL10 expression was determined by measuring the relative light units (RLUs) in a luminometer using QUANTI-Luc™, a Lucia luciferase detection reagent. Fold induction was calculated by taking into account the non-induced background.

Evaluation of NF-κB expression (SEAP reporter)

Induction of the SEAP reporter in the THP1-Blue™ KI-IP10 cells, due to the activation of the NF-κB pathway, in response to various agonists including STING ligands (cyclic dinucleotides (CDNs)) and type I IFNs has been assessed. There is a strong activation of the NF-κB pathway by STING ligands such as 2'3'- cGAMP (**Figure 3**). Furthermore, there is independent expression of the SEAP reporter by TNF-α and this allows it to be used for cross-sample normalization when studying the expression of CXCL10 (Lucia luciferase reporter) across multiple conditions.

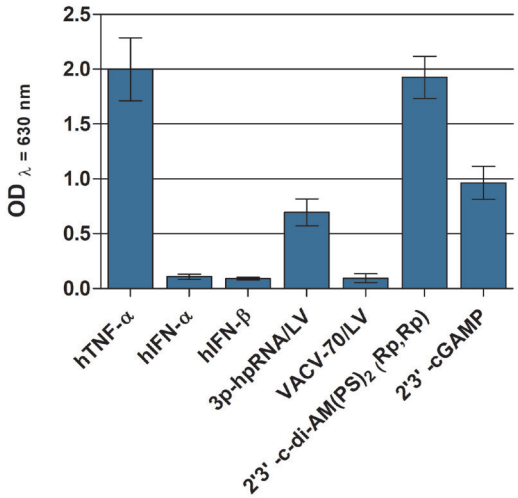

Figure 3: THP1-Blue™ KI-IP10 cells were stimulated with human (h)TNF-α (10 ng/ml), hIFN-α (1 x 10^4 U/ml), hIFN-β (1 x 10^4 U/ml), 3p-hpRNA/ LyoVec™ (LV)(1 µg/ml), VACV-70/LyoVec™ (LV)(1 µg/ml), 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) (30 µg/ml), and 2'3'-cGAMP (30 µg/ml). After 24h, NF-κB activation was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 630nm.