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iLite™ Reporter Gene Assay for Quantification of the Activity of Anti-IL-23 and Anti-IL-23 Neutralizing Antibodies

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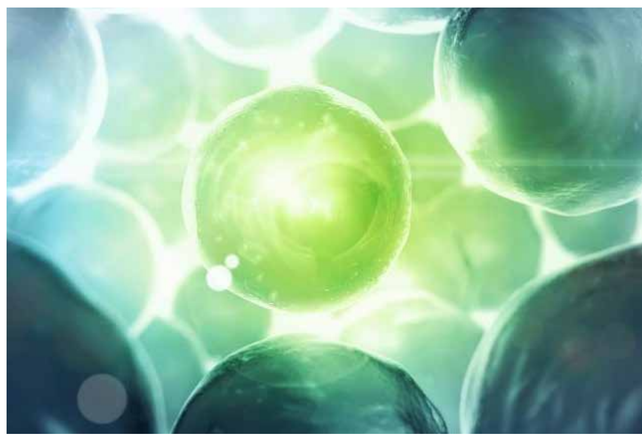

Introduction

Interleukin 23 (IL-23) is a heterodimeric pro-inflammatory cytokine that shares a common p40 subunit and a common receptor chain with IL-12. Both cytokines exert however distinct non-redundant biological functions. Conventional assays for IL-23 activity are based on the ability of IL-23 to support the proliferation of cell lines such as the IL-2 dependent human T-cell line Kit 225, which has been reported to partially lose dependence on IL-23, rendering the routine use of such assays problematic.

The IL-23 Gene reporter cell line

In order to reduce assay time from 2 days or more required for a conventional IL-23 bioassay, to 6 hours or less, and to obviate non-specific activation by other cytokines or growth factors with overlapping biological activity, a reporter gene assay was established using the avian B-cell line DT-40 that does not require IL-23 or other human cytokines in order to proliferate and that is unresponsive to the growth factors present in human serum.

Thus, DT-40 cells were co-transfected with the IL-23R and IL-12Rb1 receptor chains together with a STAT5 expression vector, a STAT5 responsive Firefly luciferase (FL) reporter gene construct, and the Renilla luciferase (RL) normalization gene under the control of a constitutive promoter (Figure 1).

Assay performance

The IL-23 responsive cells (DT4023L) exhibit a 30-fold or greater increase in IL-23 induced FL activity 5 hours after treatment with increasing concentrations of IL-23 (Figure 2). The IL-23 responsive cells provide a highly sensitive assay for the quantification of IL-23 activity with an EC50 of 1.0 ng/ml or less and a lower limit of quantification of 100 pg/ml (Figure 2). The assay is also highly selective with no detectable cross-reactivity with IL-12 (Figure 2) even though both cytokines share a common p40 chain and a common receptor chain.

Assay normalization

Both FL and RL activity are read sequentially in the same well of a micro-titer plate using Dual Glo™ (Promega).

This allows IL-23 induced FL activity to be normalized with respect to the constitutive expression of RL activity thus rendering the assay independent of cell number and providing a very effective means for compensating for serum matrix effects.

Applications

The IL-23 responsive reporter gene cell line (DT4023L) can be used as both a sensitive and specific assay for the quantification of IL-23 activity or for the quantification of the potency of anti-IL-23 or anti-p40 monoclonal antibodies (Figure 3).

This cell line also provides a very effective means of quantifying the neutralizing antibody response against such products in human serum in the absence of serum matrix effects.

Conclusion

- IL-23 responsive cell line: quantification of IL-23, anti-IL-23 or anti-p40 activity within 6 hours
- IL-23 response without any response from IL-12
- Normalized readout: eliminates serum matrix effects & renders assay results independent of cell number
- Assay-Ready Cells available for for 96 well plates
- Detailed assay description is available.

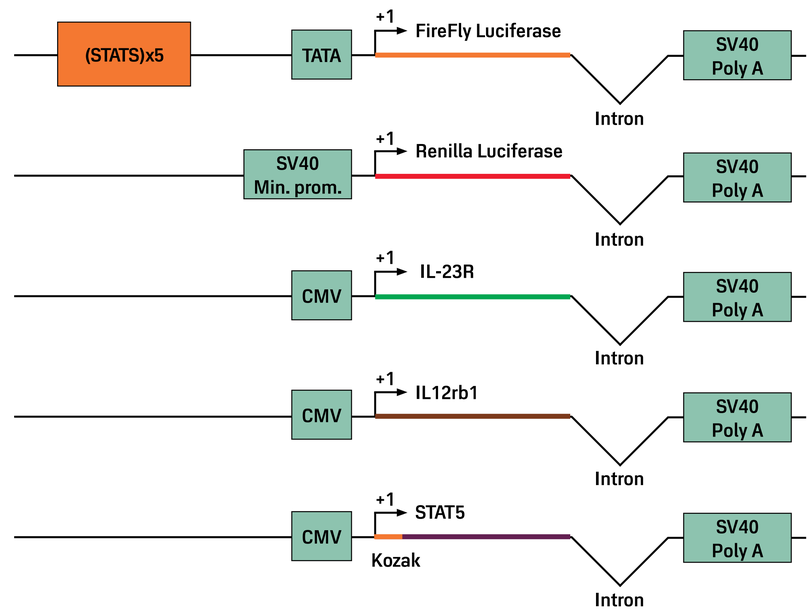

Figure 1. Molecular constructs of the IL-12 Reporter Gene Cell Line.

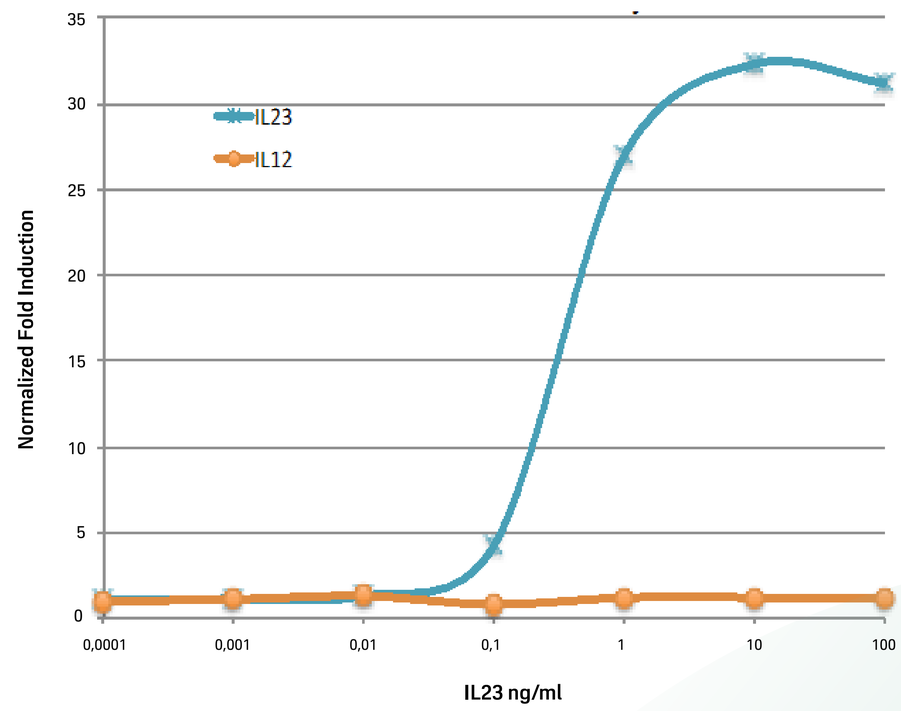

Figure 2. Response curve for the *iLite*™ IL-23 responsive Reporter Gene cell line, showing no cross-reactivity with IL-12.

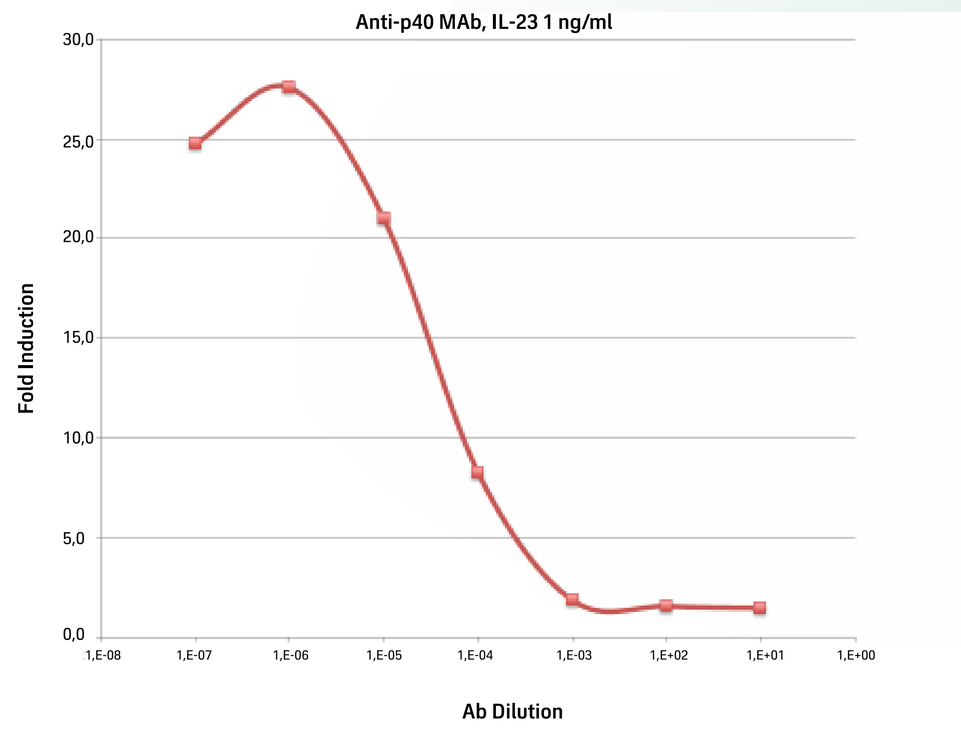

Figure 3. Quantification of an anti-p40 monoclonal antibody, using the *iLite*™ IL-23 responsive reporter cell line.