iLite® TNF-alpha

ASSAY READY CELLS

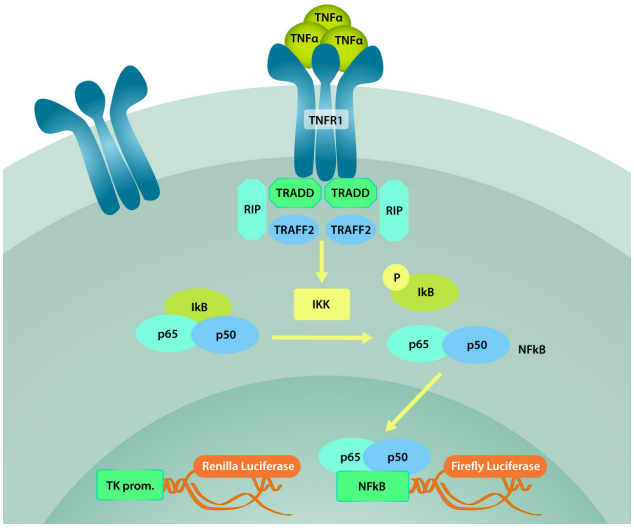
The *iLite* TNF-alpha Assay Ready Cells provide a means of directly quantifying both the potency and neutralizing antibody response to TNF-alpha antagonists of diverse structure in the same assay, making it an outstanding tool for biosimilar development.

TNF-alpha, a pro-inflammatory cytokine, is a key player in many inflammatory diseases such as rheumatoid arthritis, IBD and psoriasis. As a result, therapeutic antibodies which specifically block TNF-alpha are commonly used for treating inflammatory disorders.

Designed for use in anti-TNF-alpha drug activity and neutralizing antibody assays, the *iLite* TNF-alpha Assay Ready Cells are genetically engineered reporter gene cells that respond specifically to TNF-alpha through a luminescent readout, in a highly sensitive and reproducible manner. The use of a unique second reporter gene readout allows for normalization of results, effectively adjusting for serum matrix effects.

Measuring the functional activity of TNF-alpha, the assay can be used for both potency and immunogenicity assessments of TNF-alpha inhibitors in the same assay. As such, the *iLite* TNF-alphaα Assay Ready Cells also provide a means of directly comparing a biosimilar and innovator product in the same assay.

- Easy and fast assay format, completed within 4 hours
- Highly sensitive
- 10x fold induction
- Measures anti-TNF-alpha potency and immunogenicity in the same assay



Schematic overview of the TNF-alpha signal transduction pathway, engineered to give highly specific results.

***iLite*® TNF-alpha Assay Ready Cells**	
Product code	BM3044
Format	Assay Ready Cells

Related Products

BM4023	*iLite*® IL-23 Assay Ready Cells
BM4012	*iLite*® IL-12 Assay Ready Cells
BM4050	*iLite*® GM-CSF Assay Ready Cells
BM3049	*iLite*® Type I IFN Assay Ready Cells

Complementary Products

BM3133	*iLite*® TNF-alpha (16 ng/ mL)
BM3136	*iLite*® Infliximab NAb positive control
BM3159	*iLite*® Adalimumab NAb positive control
BM3177	*iLite*® Etanercept NAb positive control
BM3132	*iLite*® Diluent A
BM3134	*iLite*® Diluent B
BM3139	*iLite*® Diluent C
BM3135	*iLite*® Reagent BLANK

Application	*iLite* TNF-alpha Assay Ready Cells can be used for measurements of anti-TNF-alpha drug activity and functional TNF-alpha as well as presence of anti-TNF-alpha drug NAbs. The following application notes are available: • Quantification of anti-TNF-alpha drug activity • Determination of TNF-alpha inhibitor neutralizing antibodies • Quantification of functional TNF-alpha
Incubation time	Drug Assays 30 min + 3 hours NAb Assays 30 min + 30 min +3 hours
Detection system	Luminescence
Availability	Research Use Only (RUO)*

**These products are intended for professional research use only. The data and results originating from using the products, should not be used either in diagnostic procedures or in human therapeutic applications.*

In accepting delivery of iLite® Assay Ready Cells the recipient agrees not to sub-culture these cells, attempt to sub-culture them or to give them to a third party, and recipient is only to use them directly in assays. The iLite® cell-based products are covered by patents which are the property of Svar Life Science AB and any attempt to reproduce the delivered iLite® Assay Ready Cells would constitute an infringement.

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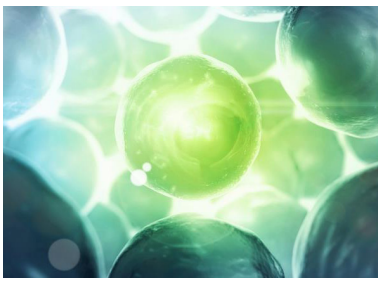
iLite® Reporter Gene Assay for the Quantification of the Activity and Neutralizing Antibody Response to TNF-α Antagonists

Christophe Lallemand and Michael G. Tovey. Biomonitor SAS, Villejuif, France



Introduction

Antagonists of tumor necrosis factor alpha (TNF-α) are used widely for the treatment of a number of chronic inflammatory or autoimmune diseases such as rheumatoid arthritis (RA), psoriasis, and Crohn's Disease. Such antagonists include; infliximab (Remicade®), a chimeric monoclonal antibody against TNF-α, adalimumab (Humira®) and golimumab (Simponi®), fully human monoclonal anti-TNF-α antibodies, etanercept (Enbrel®), a fusion protein comprising the p75 chain of the TNF-α receptor and the Fc moiety of human IgG1, and certolizumab (Cimzia®), pegylated Fab' fragments of a humanized anti-TNF-α monoclonal antibody.

Method

A cell-based assay has been developed for the quantification of the activity of TNF-α antagonists that signal through the NFkB pathway (Figure 1) based on human erythro-leukemic K562 cells transfected with a 5 x tandem repeat of a non-canonical NFkB recognition sequence regulating expression of the Firefly luciferase reporter-gene (Figure 2).

The use of a TNF-α specific reporter gene construct (Figure 2) together with cells engineered not to respond to other factors that signal through the NFkB pathway renders the assay specific for TNF-α (Figure 3).
The assay is rapid and can be completed within 4 hours, is simple to perform (Figure 4), highly sensitive (Figure 5) and yields a dynamic range of some 90-fold (Figure 3).

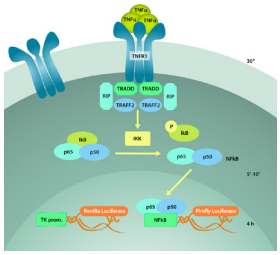


Figure 1. TNF-α signal transduction pathway.

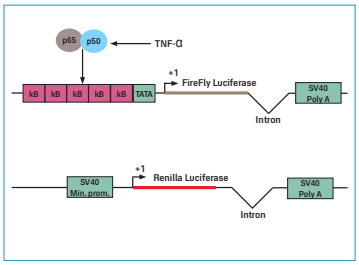


Figure 2. TNF-α normalized reporter-gene construct.

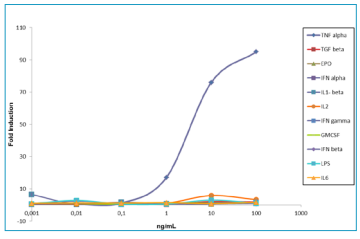


Figure 3. Specificity of the response of the *iLite* TNF-α responsive cells..

Characteristics	*iLite* TNF-α responsive cells
Time of incubation	4 hours
Normalization	Yes
EC50	2 ng/mL
LLQ	500 pg/mL

Figure 4. TNF-α Dose response curve - 4 hour assay, 25 000 cells/well.

Results – Sensitivity

The cells also contain a Renilla luciferase reporter gene controlled by a constitutive promoter (Figure 2). This allows TNF-α induced Firefly luciferase activity to be normalized relative to Renilla luciferase expression thus rendering results independent of cell number (Figure 5) and providing an efficient means for correcting for serum matrix effects (data not shown).

References

1. Lallemand et al. J.Immunol. Methods. 373: 229-239, 2011

Results - Quantification of Potency & NAbs with the same Assay

The *iLite* TNF-α responsive reporter gene cell line provides a means of directly quantifying both the potency (Figure 6) and neutralizing antibody response to TNF-α antagonists of diverse structure in the same assay. The *iLite* TNF-α responsive reporter gene cell line also provides a means of directly comparing both the potency and neutralizing antibody response of a biosimilar and innovator product in the same assay (data not shown).

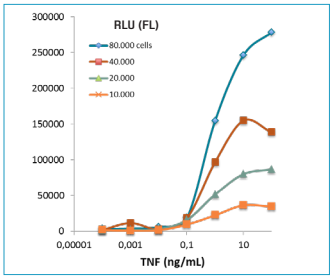


Figure 5a. Relationship between TNF-α induced FL expression and cell number without normalization.

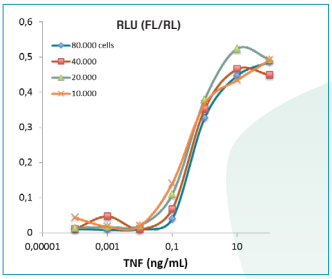


Figure 5b. Relationship between TNF-α induced FL expression and cell number using normalization with RL expression.

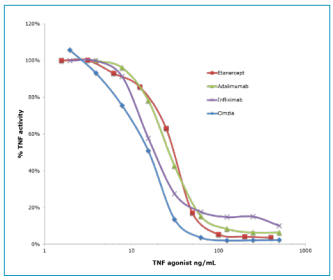


Figure 6. Quantification of the activity of TNF-α antagonists using *iLite* TNF-α responsive reporter gene cell cells.

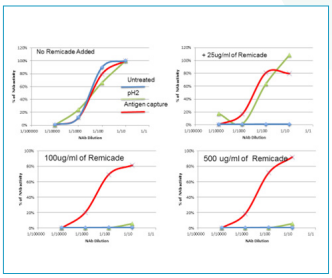


Figure 7. Quantification of the activity of anti-Remicade NAbs in presence of various concentrations of drug before and after antigen capture.

Results - Robustness

The use of TNF-α covalently bound to a solid support provides an efficient means of removing residual drug from patient sera and rendering the assay drug tolerant. Results presented here illustrate the robustness of the assay for the routine quantification of both drug activity and anti-drug neutralizing antibodies in serum samples from patients with inflammatory disease treated with TNF-α antagonists even in the presence of high concentrations of drug (Figure 7).

Conclusion

- **The *iLite* TNF-α responsive reporter gene assay provides a precise, sensitive and rapid means of quantifying both drug activity and neutralizing anti-drug antibodies present in serum samples without serum matrix effects.**
- **Thus, the reporter gene assay described herein is ideally suited for high throughput quantification of residual drug activity and anti-drug NAb levels in samples of serum from patients with inflammatory disease treated with TNF-α antagonists.**
- **This single assay is applicable to the quantification of the activity or NAb response to a variety of different TNF-α antagonists including innovator products and biosimilars.**
- **Furthermore, the simple antigen capture procedure described herein allows neutralizing anti-drug antibodies to be detected even in sera containing high concentrations of drug and provides a means of detecting the onset of a NAb response prior to the clinical symptoms of drug resistance.**