

Reproducible, MoA-reflecting Reporter-based Bioassays to Enable Drug Development of Biosimilars and Biobetters

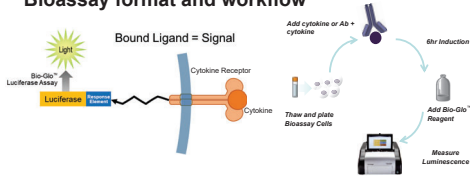


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1. Introduction

Cytokines and growth factors can be described as small immunomodulatory proteins secreted by a wide variety of cells including fibroblasts, endothelial and stromal cells, whose role it is to regulate surrounding cells in an autocrine, paracrine or endocrine fashion. Immunocytokines represent a promising class of activators of the immune system, with the potential to be used alone or in combination with other therapeutic modalities. Many are currently FDA approved therapy agents (e.g. IFN, IL-2, and Epo), while others are targets for approved biological blocking therapies to support treatment of a variety of diseases. Examples of cytokine blocking agents include basiliximab (IL-2R), tocilizumab and sarilumab (IL-6R), siltuximab (IL-6), ustekinumab and its biosimilars (IL-12/IL-23 p40), secukinumab (IL-17A), bevasizumab (VEGF), and denosumab (RANKL). Many more are in development and trials as biosimilars and biobetters. IL-2 and IL-15 are still clinically important cytokines as researchers look to improve potency, patient tolerance, and response by developing new molecules with sustained and targeted activities. We have developed luciferase reporter bioassays which individually can be used for the quantitation of a variety of cytokines and growth factors including IL-2, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17, IL-23, TNF α , TGF β , BCMA, TPO, VEGF, and RANKL using respective mechanism of action pathways. The bioassay format is based on thaw-and-use cells, eliminating the need to establish and pre-culture cytokine responsive cell lines which provides the benefits of convenience, reproducibility, and transferability. We demonstrate these assays measure cytokine response and inhibition with blocking drugs or potency changes from stressed samples. In summary, these reporter-based bioassays provide valuable tools for the development, stability testing, and potency determination in the manufacture of cytokine biosimilars and biobetters.

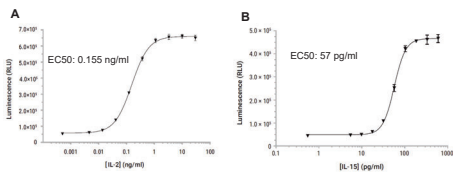
Bioassay format and workflow



Each bioassay contains an engineered cell line with the appropriate receptor and pathway inducible luciferase reporter.

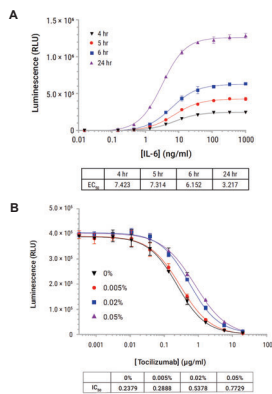
Most cytokine or cytokine blocker assays can be completed within one working day using the thaw-and-use cell assay format.

2. IL-2 and IL-15 Bioassays



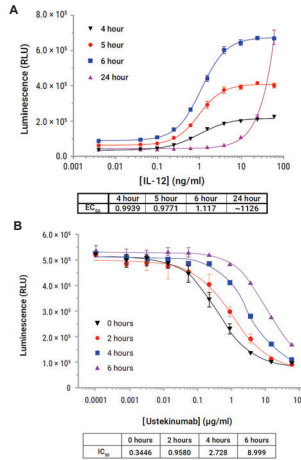
- The IL-2 Bioassay is responsive to recombinant IL-2. Following a 6-hour induction, the EC₅₀ was 0.155ng/ml, with a fold induction of 12.
- The IL-15 Bioassay is responsive to recombinant IL-15. Following a 6-hour induction, the EC₅₀ was 57 pg/ml, with a fold induction of 10.5.

3. IL-6 Bioassay



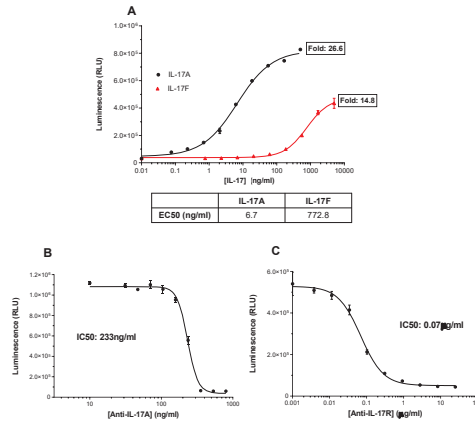
- IL-6 response following a range of incubation times with recombinant IL-6. Data was generated using thaw-and-use cells.
- The IL-6 Bioassay indicates stability. Tocilizumab was treated with various concentrations of hydrogen peroxide (0–0.05%) for 18 hours at 26°C prior to use in the IL-6 Bioassay.

4. IL-12 Bioassay



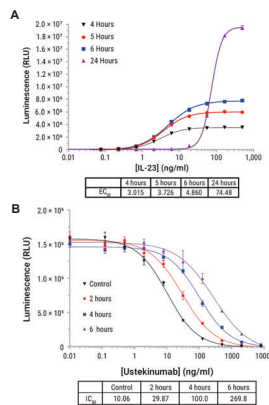
- IL-12 response following a range of incubation times with recombinant IL-12. Data was generated using thaw-and-use cells.
- The IL-12 Bioassay indicates stability. Ustekinumab was heat treated at 65°C for 0–6 hours prior to use in the IL-12 Bioassay.

5. IL-17 Bioassay



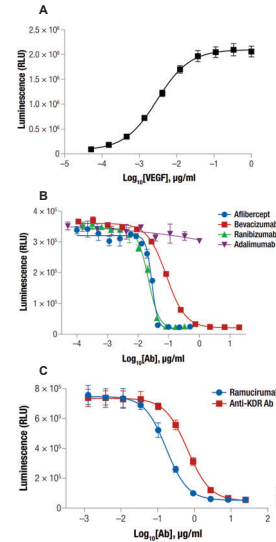
- IL-17A or IL-17F response following 6-hour induction with recombinant cytokine.
- Blocking of IL-17A activity using anti-IL-17A antibody.
- Blocking of IL-17 activity using anti-IL-17 receptor antibody.

6. IL-23 Bioassay



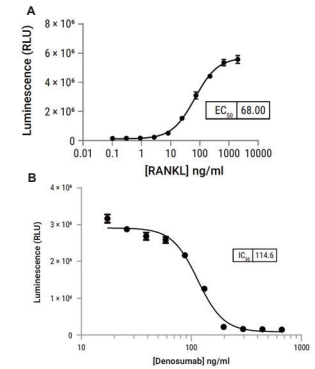
- IL-23 response following a range of incubation times with recombinant IL-23. Data was generated using thaw-and-use cells.
- The IL-23 Bioassay indicates stability. Ustekinumab was heat treated at 65°C for 0–6 hours prior to use in the IL-23 Bioassay.

7. VEGF Bioassay



- VEGF response using thaw-and-use cells following 6-hour induction.
- The bioassay can be used to measure the activity of antibodies against VEGF. Assay is specific, showing no response to Adalimumab (anti-TNF α).
- The bioassay can be used to measure the activity of antibodies against the VEGF receptor (KDR) in the presence of VEGF.

8. RANKL Bioassay



- RANKL response following a 6-hour incubation with recombinant RANKL.
- Blocking of RANKL activity using anti-RANKL antibody.

9. Conclusions

The demand for biosimilars, and bioassays for biosimilars, is increasing dramatically as the patent cliff approaches for many blockbuster biologics. The FDA approval of drugs such as tocilizumab to treat CAR-T induced severe cytokine storm demonstrates the great potential of cytokine blockers in the treatment of cancer and auto-immune diseases.

- Reporter based bioassays can measure relative potency for antibody biologics, and detect potency changes for stressed antibody samples
- Thaw-and-use cells bring the benefit of convenience, low day-to-day variation, and easy lab-to-lab assay transfer
- Cell-based reporter bioassays for IL-2, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17, IL-22, IL-23, TNF α , TGF β , BCMA, TPO, VEGF, and RANKL have been developed that can be used to quantitate specific cytokine response or blocker inhibition

In summary, the reporter-based assay portfolio for biosimilars provides a valuable tool for similarity and biobetter study, stability testing, and lot release in biosimilar drug manufacturing.