Introduction

Interleukin-2 (IL-2), first described in 1976 as T-cell growth factor, is a 15kDa glycoprotein produced primarily by activated T cells. Aldesleukin is FDA approved for the treatment of renal cell carcinoma (1992) and metastatic melanoma (1998). Aldesleukin is recombinant IL-2 and differs from its natural form by lacking glycaminoglycans (SGS derived) and two amino acid changes. Its antitumor efficacy is achieved by increased proliferation of natural killer cells, lymphokine activated killer cells, and other cytotoxic cells.

Interleukin-15 (IL-15) shares structural similarity to IL-2 and is expressed on a variety of cell types including monocytes, macrophages, dendritic and epithelial cells. Both IL-2 and IL-15 promote proliferation and differentiation of NK cells, and T and B cells. IL-15 is a membrane associated cytokine, in contrast to IL-2 which acts as a soluble molecule, and is involved in the persistence of CD8+ T cells. IL-15 plays an important role in the immune response as well as autoimmunological diseases.

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. Peptide, superagonists, immunocomplexes, and immunotoxins are current strategies in clinical development.

We have developed a luciferase reporter bioassay which can be used for the quantification of both IL-2 and IL-15 using the cytokine’s mechanism of action pathway. The bioassay format is based on thaw-and-use cells, eliminating the need to establish and pre-culture traditional IL-2 responsive cells such as CTLL-2. This format also provides the benefit of convenience, reproducibility, and transferability. Quantitative measurement of IL-2 or IL-15 using this reporter bioassay is complete within 7 hours, versus 2-3 days using traditional proliferation assay protocol can be performed. We show the bioassay is stability-indicating using heat stressed Aldesleukin samples. And finally the cell line demonstrates stability and specificity.

**IL-2/3/15 Bioassay Protocol**

1. Thaw and Use (Protocol)
2. IL-2 or IL-15
3. Induction

**4. Aldesleukin stability indicating**

<table>
<thead>
<tr>
<th>IL-15 (ng/mL)</th>
<th>Fold Induction</th>
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<tbody>
<tr>
<td>0.00</td>
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<tr>
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IL-2/3/15 Bioassay Cells. Thaw-and-Use were thawed, diluted and plated into a solid white 96-well plate. Serial dilutions of IL-2 (Proleukin® 0.00-10) or IL-15 (Prometheus, 1.0mg/ml) were prepared in Assay Buffer and added. Plate was incubated for an additional 6 hours, after which samples were treated with Bio-GloTM Luciferase Assay Reagent and luminescence was measured in Spectramax®NOS (Molecular Devices, San Jose, CA). For proliferation assay comparison, CTLL-2 cells were seeded into 96-well plate and cultured 48 hours in the absence of IL-2. Serial dilutions of IL-2 were added and plated incubated an additional 48 hours before the addition of CellTiter-Glo® to measure viability.

**5. The Bioassay is Receptor Specific**

Aldesleukin (Prometheus, 1.1ng/ml undiluted) or a research grade equivalent (Milteny, 20ug/ml with BSA carrier) or recombinant IL-2 (PropeTech) were heat stressed at various temperatures and durations prior to being tested using thaw-and-use cells in a 5 hour incubation experiment.

**6. Receptor expression cell line passage stability**

**7. Functional cell line passage stability**

**8. Conclusions**

- The Promega IL-2/15 Bioassay responds to IL-2 and IL-15 in a dose-dependent manner, using a thaw-and-use format.
- The Thaw and Use format eliminates pre-culturing of cells, facilitates transferability and provides reproducible results.
- The reporter bioassay demonstrated a lower EC50 with better replicates than a traditional CTLL-2 proliferation assay, and is completed within one day.
- The bioassay was able to detect aldoseleukin heat-stressed EC50 changes following treatment for as little as 2 hours.
- The cell line is demonstrated to be stable as judged by functional fold induction and surface receptor expression.

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March, 2018

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