A Cell-based Luciferase Reporter Bioassay for Interleukin-2 and Interleukin-15 testing

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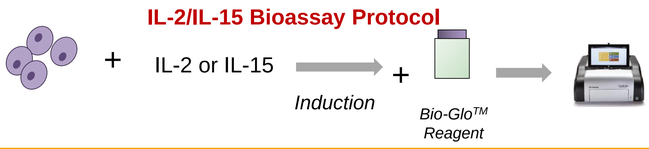
1. Introduction

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 glycosylation (E.coli 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

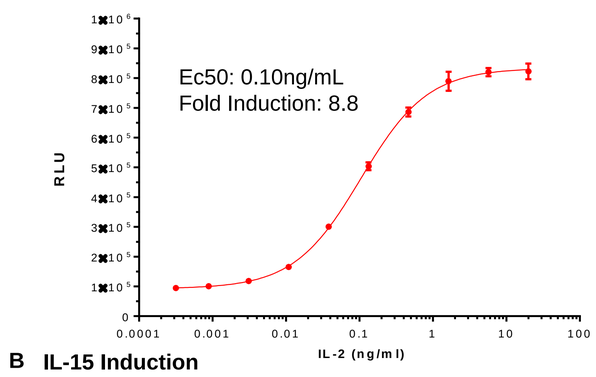
Interleukin-15 (IL-15) shares structural similarity to IL-2 and is expressed on a variety of cell types including monocytes, macrophages, dendritic and epithelial cells. Both IL-2 and IL-15 promote proliferation and differentiation of NK cells, and T and B cells. IL-15 is a membrane associated cytokine, 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 innate immune response as well as autoimmune inflammatory 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. PEGylation, superagonists, immunocomplexes, and immunocytokines are current strategies in clinical development.

We have developed a luciferase reporter bioassay which can be used for the quantitation 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 in less than 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.



2. IL-2 & IL-15 Response in Thaw-and-Use assay format



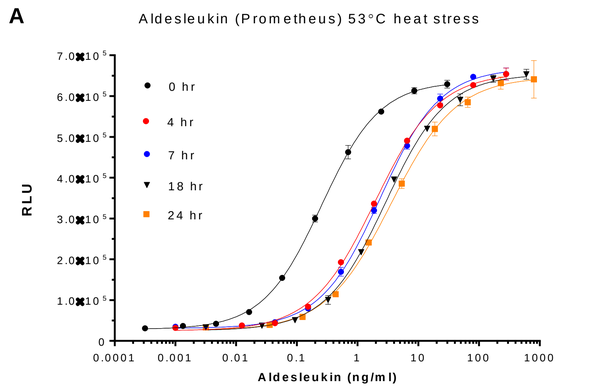
IL-2/IL-15 Bioassay Cells, Thaw-and-Use were thawed, diluted and plated into a solid white 96-well plate. Serial dilutions of IL-2 (Peprotech #200-2) or IL-15 (Peprotech, Rocky Hill NJ. #200-15) were prepared in Assay Buffer and added. Plate was incubated for an additional 6 hours, after which samples were treated with Bio-Glo™ Luciferase Assay Reagent and luminescence was measured with GloMax Discover.

3. Comparison to CTLL-2 proliferation assay

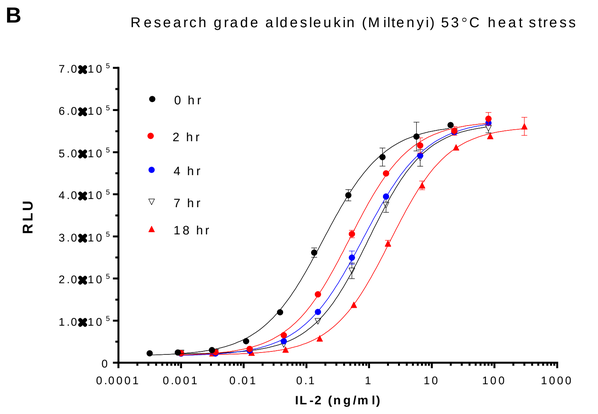
	Reporter Assay	Proliferation
Ec50 (ng/mL)	0.24	0.66

IL-2/IL-15 Bioassay Cells, Thaw-and-Use were thawed, diluted and plated into a solid white 96-well plate. Serial dilutions of IL-2 were prepared and added. Plate was incubated for an additional 6 hours, after which samples were treated with Bio-Glo™ Luciferase Assay Reagent and luminescence was measured in SpectraMax® M5e (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 plate incubated an additional 48 hours before the addition of CellTiter-Glo® to measure viability.

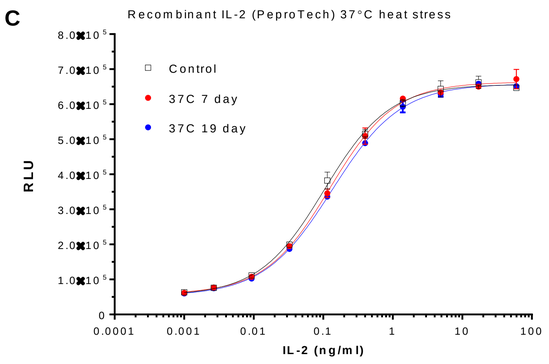
4. Aldesleukin stability indicating



	0hr	4hr	7hr	18hr	24hr
Ec50 (ng/mL)	0.26	1.94	2.35	2.90	3.56



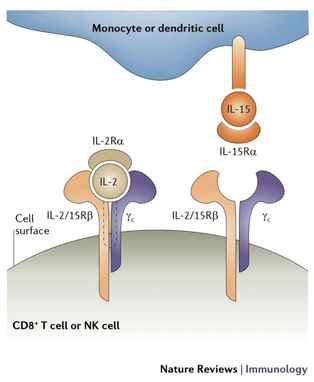
	0hr	2hr	4hr	7hr	18hr
Ec50 (ng/mL)	0.18	0.49	0.81	0.99	2.2



	Control	37C 7 days	37C 19days
Ec50 (ng/mL)	0.10	0.12	0.13

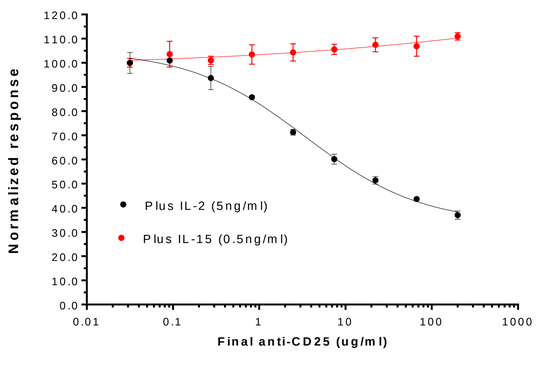
Aldesleukin (Prometheus, 1.1mg/ml undiluted) or a research grade equivalent (Miltenyi, 20ug/ml with BSA carrier) or recombinant IL-2 (PeproTech) were heat stressed at various temperatures and durations prior being tested using thaw-and-use cells in a 6 hour induction experiment.

5. The Bioassay is Receptor Specific



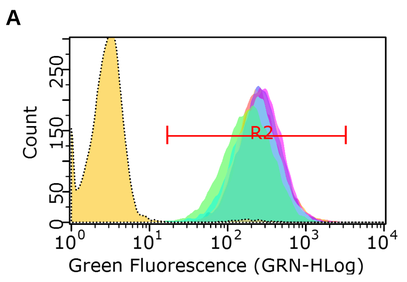
Lee and Margolin, *Cancers* 2011, 3(4), 3856-3893.

Specificity with IL-2Rα (CD25) receptor block

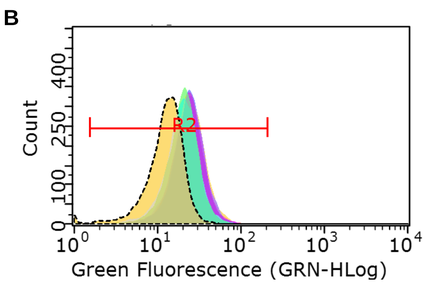


Thaw-and-use cells were pre-treated with a titration of receptor blocking anti-IL-2Rα (CD25) monoclonal prior to addition of either IL-2 (5ng/ml final) or IL-15 (0.5ng/ml final). Specific receptor blocking of IL-2 response observed, with no impact on IL-15 activity.

6. Receptor expression cell line passage stability



Passage	MFI for IL-2Rα (CD25)
Pass 9	185
Pass 17	229
Pass 24	260
Pass 32	280
Pass 39	254
Pass 46	269

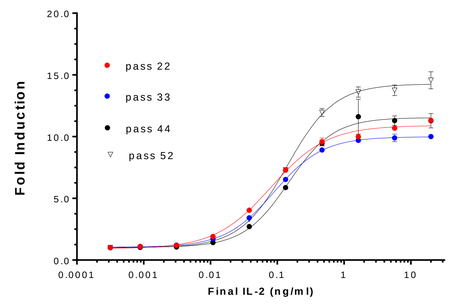


Passage	MFI for IL-2Rβ (CD122)
Pass 8	20
Pass 16	20
Pass 23	24
Pass 31	23
Pass 39	23
Pass 45	24

Flow cytometry surface staining of IL-2Rα (CD25) or IL-2Rβ (CD122) of IL-2/IL-15 Bioassay cells across passage 8-46 demonstrating consistent mean fluorescent intensity across passages. Cells maintained and passaged 3x per week.

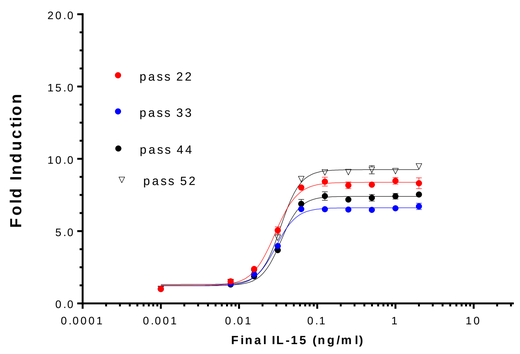
7. Functional cell line passage stability

A IL-2 Induction



	Passage 22	Passage 33	Passage 44	Passage 52
Ec50 (ng/mL)	0.08	0.109	0.15	0.15
Fold Induction	10.9	10.0	11.5	14.3

B IL-15 Induction



	Passage 22	Passage 33	Passage 44	Passage 52
Ec50 (ng/mL)	0.03	0.03	0.03	0.03
Fold Induction	8.4	6.6	7.4	9.3

Functional cell line stability of continuously growing IL-2/IL-15 Bioassay Cells. IL-2 and IL-15 response demonstrates passage stabilization (fold induction and EC50) across passage 22-52

8. Conclusions

- The Promega IL-2/IL-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 aldesleukin 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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