

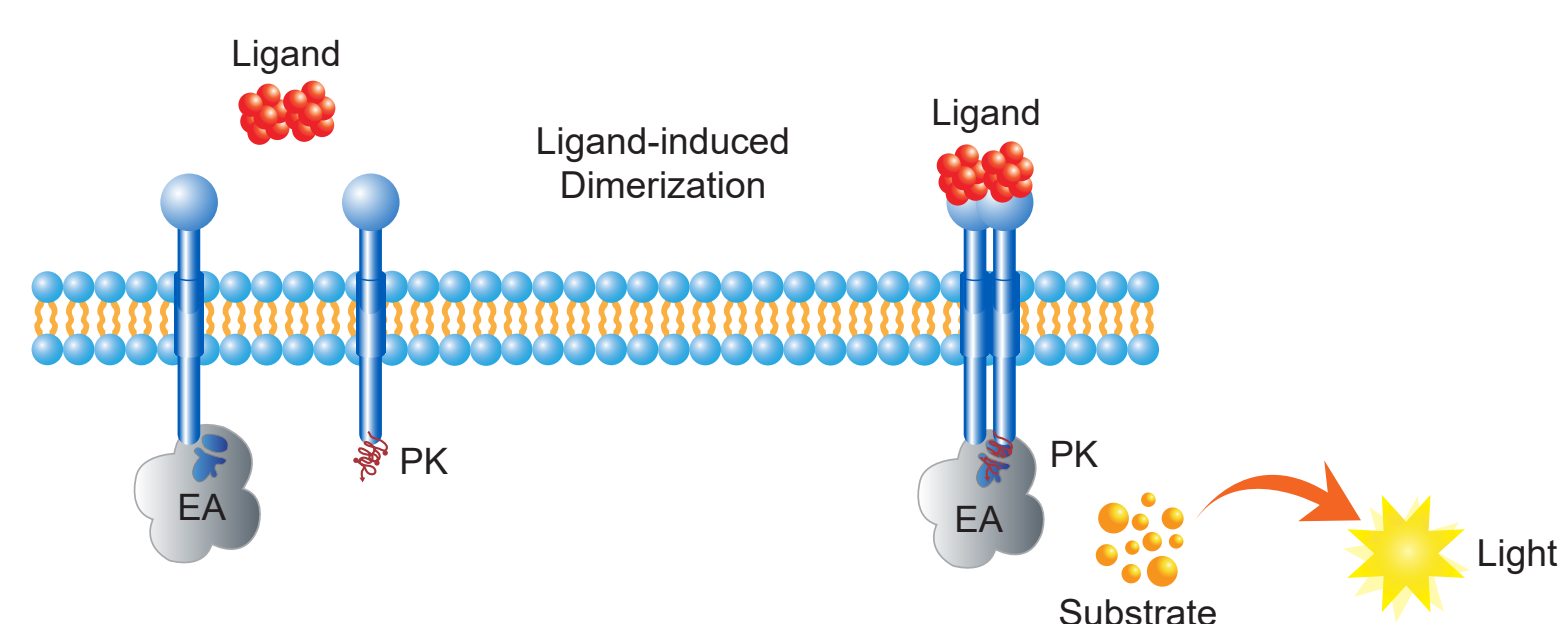
# Building Robust and Simple Cell-Based Assays for Interleukins and Their Receptors

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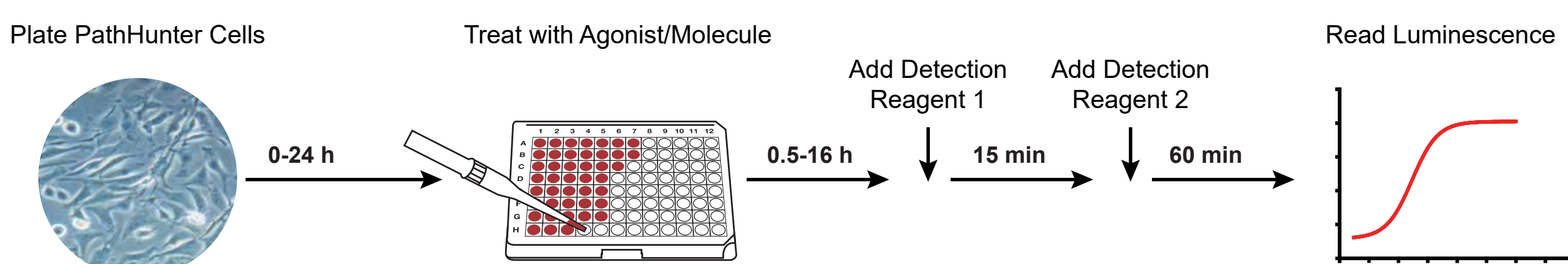
## Abstract

Ligand-induced receptor dimerization is an early functional step in receptor activation, representing the most proximal, functional readout for Interleukin receptors. It is well understood that specific signaling receptor subunits can heterodimerize with multiple high affinity receptors from the same family leading to complicated signaling cascades that are implicated in auto-immune, inflammatory, and oncogenic diseases. Here we present a novel application of our PathHunter® technology to monitor receptor-receptor interactions at the surface of intact live cells, applicable to diverse receptor types, with a specific focus on the interleukin family of receptors. The high specificity and simplicity of the assay protocol, serum tolerance, and reproducibility of these assays has enabled their use in cell-based screening, functional characterization, and QC lot release assays. Examples from our menu of assays spanning ~80% of all human interleukins will be presented, including assays for Anakinra and Tocilizumab. These robust assays have excellent reproducibility, accuracy and precision, demonstrating their suitability for use as QC lot release assays.

## Mechanism of Action-Based Functional PathHunter Interleukins Assays

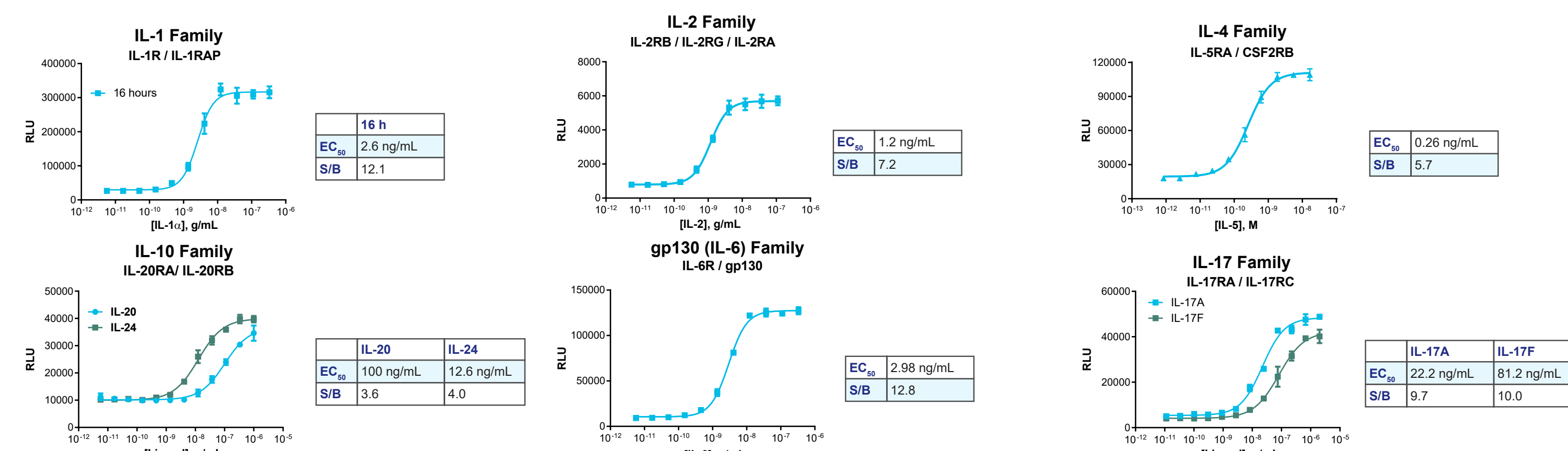


## A Simple Homogenous Protocol with Rapid Results



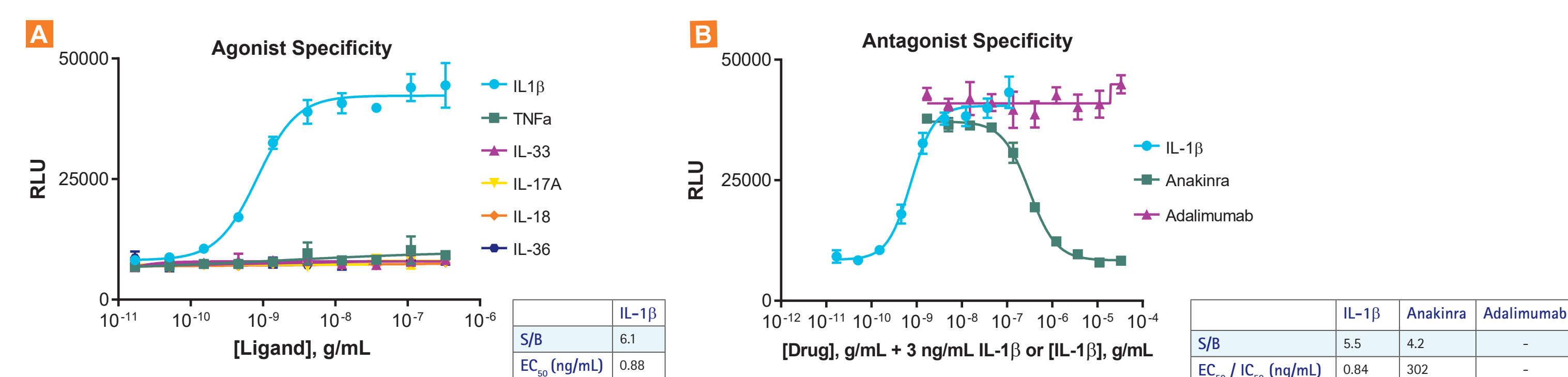
PathHunter cell-based assays use a simple homogenous protocol with rapid results. Cells are plated on a 96-well plate and incubated for 0-24 hours at 37°C. The agonist/test molecule is added to the plate and incubated for 0.5-16 hours, with specific times optimized for each assay. The detection reagents are added sequentially in two addition steps and the chemiluminescent signal can be detected on any plate-reading luminometer. PathHunter assays for use in QC lot release testing are available as continuous culture cell lines, or can be custom developed into ready-to-assay cells.

## Highly Specific Assays for Interleukin Receptors Across Multiple Families



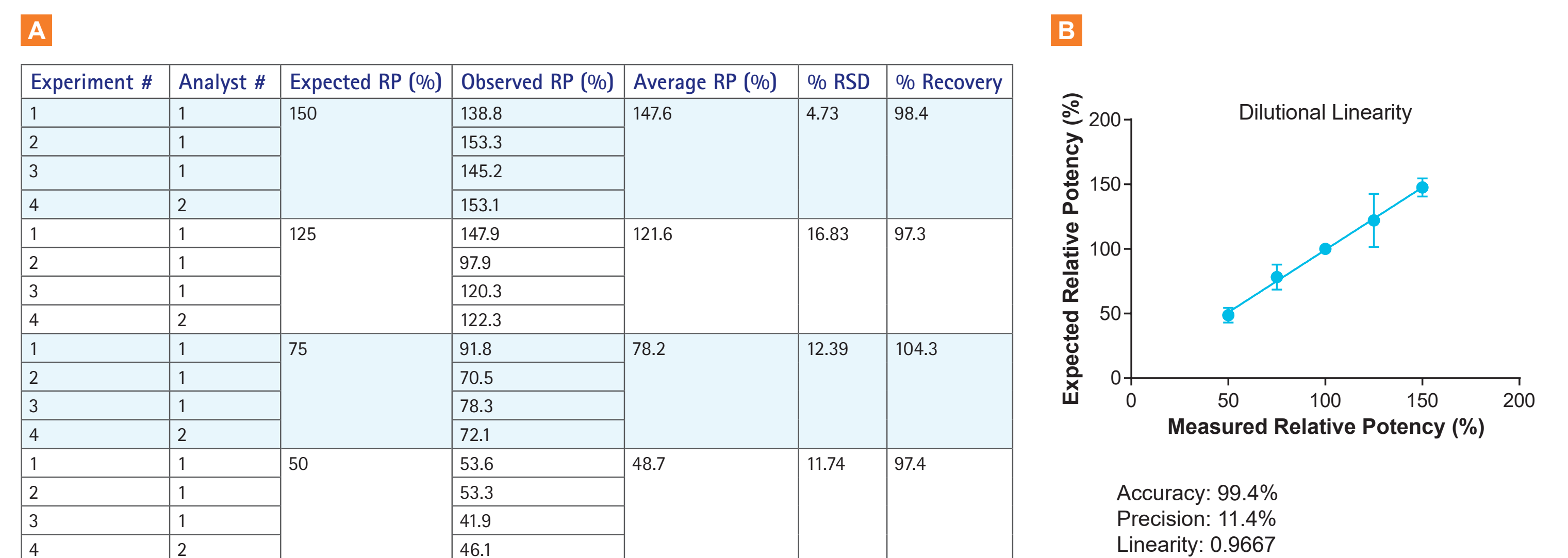
Representative examples of assays for interleukin receptors from 6 different families of interleukins/IL receptors. Each plot shows a functional dose response for the relevant ligand(s) in a given assay from the indicated family. Data plotted are mean RLU and standard deviation from at least triplicate wells for each dose. These assays are characterized by robust assay windows and high reproducibility.

## Anakinra Bioassay (IL-1RA/IL-1RAP Dimerization) Is Highly Specific



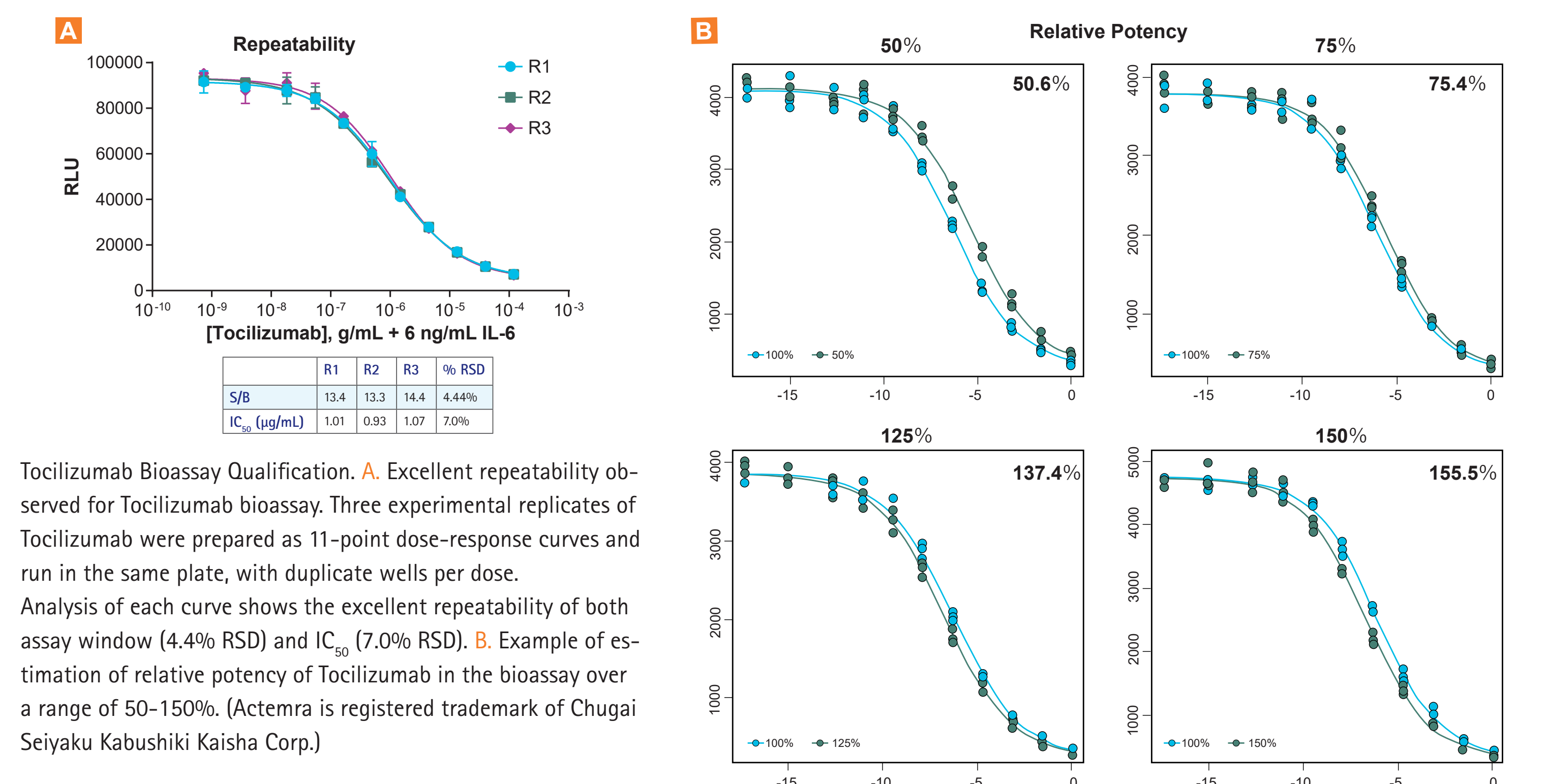
Specificity of Anakinra Bioassay. **A.** Bioassay cells expressing the IL-1 receptor (assay quantifies ligand-induced heterodimerization of the IL-1RA and IL-1RAP subunits that comprise the receptor) were treated with various ligands that mediate signaling through the NF-κB pathway, which acts downstream of multiple IL-1 family receptors. Only IL-1β induces a signal in the assay, reflecting the specificity of the assay for IL-1β. **B.** Two different drugs, Anakinra (which blocks signaling through the IL-1R by blocking IL-1β-mediated dimerization and signaling) and Adalimumab (which binds TNFα, and prevents signaling through the TNFR) were tested in the IL-1R/IL-1RAP bioassay. Only Anakinra blocks IL-1β-mediated dimerization.

## Anakinra Bioassay Qualification with Kineret®



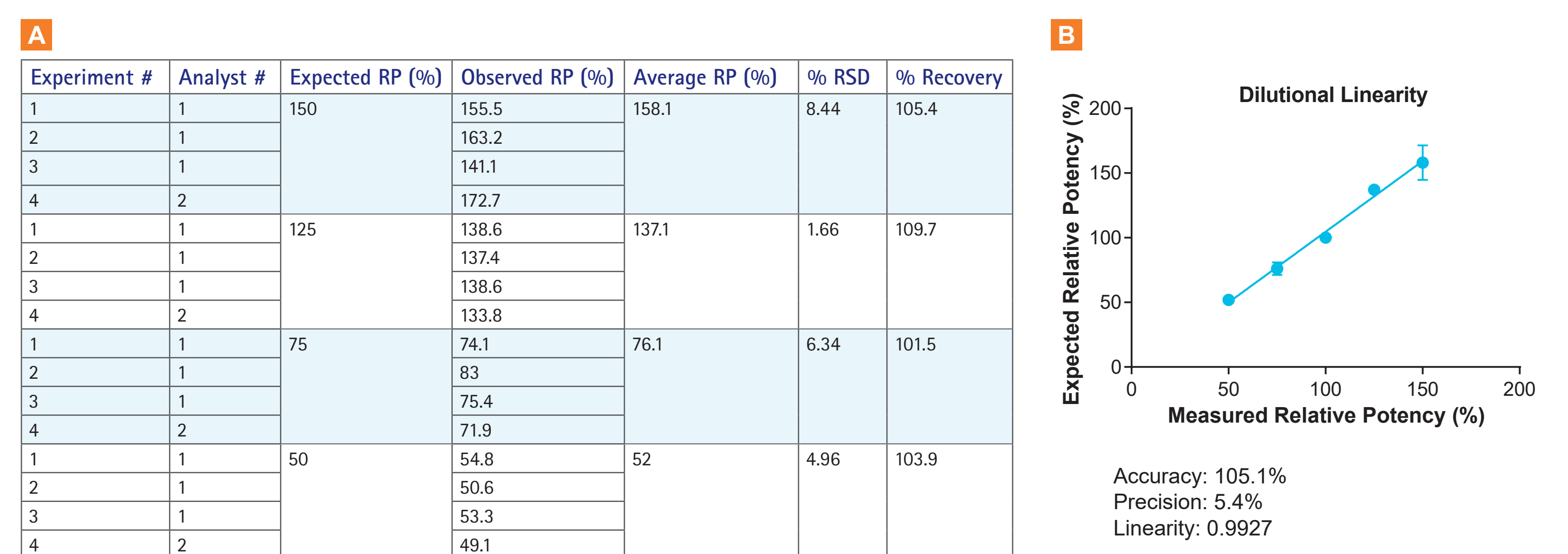
**A.** Results of Anakinra bioassay qualification over a range of 50% to 150% by two analysts. Each concentration of Anakinra was tested in 4 different experiments; at least one of those was performed by a second analyst. **B.** Linearity of the Anakinra bioassay is plotted. The Anakinra bioassay demonstrates excellent accuracy, precision, and linearity. (Kineret is registered trademark of Swedish Orphan Biovitrum AB)

## Tocilizumab Bioassay (IL-6RA/IL-6ST Dimerization) Qualification with Actemra®



Tocilizumab Bioassay Qualification. **A.** Excellent repeatability observed for Tocilizumab bioassay. Three experimental replicates of Tocilizumab were prepared as 11-point dose-response curves and run in the same plate, with duplicate wells per dose. Analysis of each curve shows the excellent repeatability of both assay window (4.4% RSD) and  $IC_{50}$  (7.0% RSD). **B.** Example of estimation of relative potency of Tocilizumab in the bioassay over a range of 50-150%. (Actemra is registered trademark of Chugai Seiyaku Kabushiki Kaisha Corp.)

## Tocilizumab Bioassay Qualification with Actemra®



**A.** Results of Tocilizumab bioassay qualification over a range of 50% to 150% by two analysts. Each concentration of Tocilizumab was tested in 4 different experiments; at least one of those was performed by a second analyst. **B.** Linearity of the Tocilizumab bioassay is plotted. The Tocilizumab bioassay demonstrates excellent accuracy, precision, and linearity.

## Summary

- Largest Menu of Ready-to-Use Assays - Save 3 to 6 months in assay development for ~ 85% of known interleukins
- Unparalleled Specificity - Screen crude biologic samples with confidence
- High Reproducibility - Precise, accurate, reproducible, and robust assay for use in QC lot release