



PathHunter[®] Tocilizumab Signaling Bioassay

Qualified with Actemra[®]

[93-1045B3-00109](#) (2-Plate Kit)

[93-1045B3-00110](#) (10-Plate Kit)

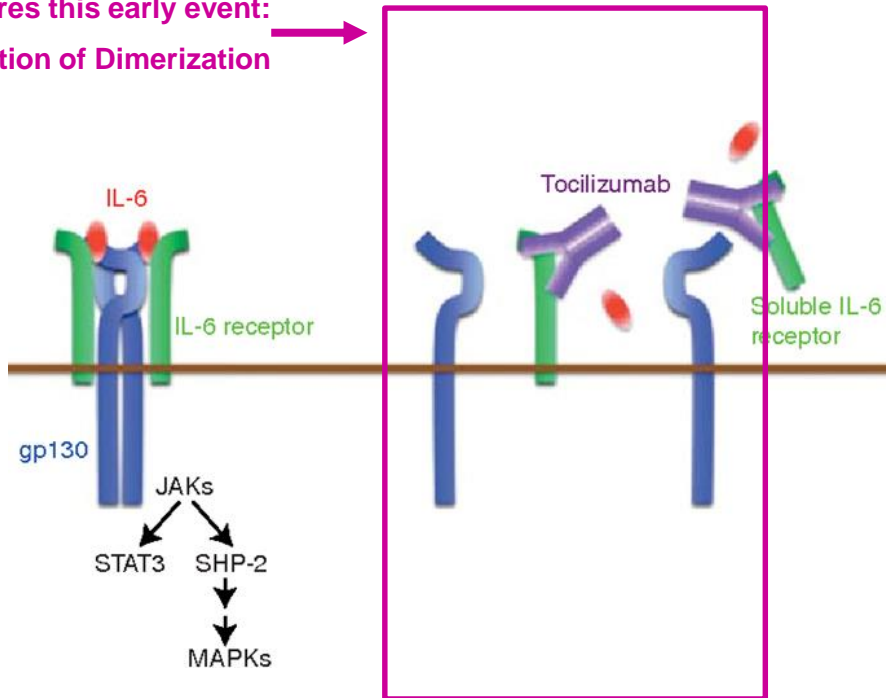
OUR EXPERTISE
IN YOUR HANDS.
DISCOVER
CONFIDENTLY.

Tocilizumab-mediated inhibition of IL6-mediated IL6R/IL6ST dimerization

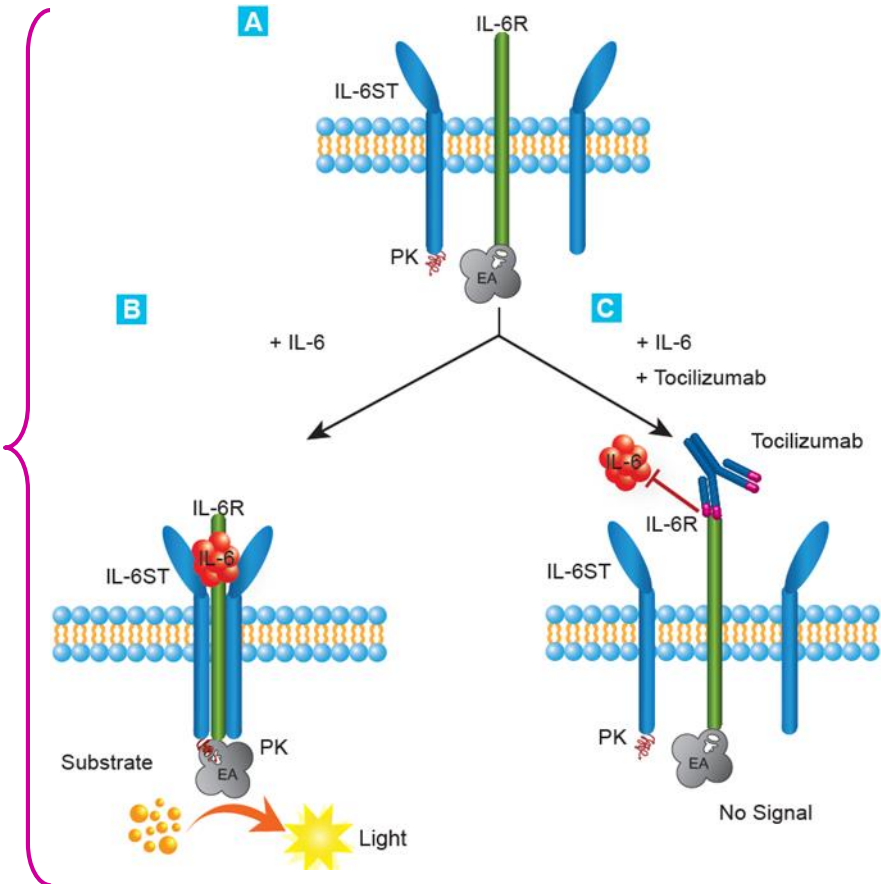
Mechanism of Action

Assay Design

Assay measures this early event:
Inhibition of Dimerization



Source: Tanaka, Toshio et al. "IL-6 in inflammation, immunity, and disease." *Cold Spring Harbor perspectives in biology* 6 10 (2014): a016295.



Chemiluminescent Detection of Anti-IL6R Antibody-mediated inhibition of IL6R/IL6ST Dimerization

PathHunter[®] Tocilizumab Bioassay Kit

Kit Components

| List of Components | 93-1045B3-00109 | 93-1045B3-00110 |
|---|-----------------|-----------------|
| PathHunter U2OS IL6R/IL6ST Bioassay Cells (No. of vials) | 2 | 10 |
| PathHunter Bioassay Detection Kit (No. of datapoints) | 200 | 1,000 |
| Detection Reagent 1 (mL) | 2 | 10 |
| Detection Reagent 2 (mL) | 8 | 40 |
| AssayComplete™ Cell Plating 5 Reagent* | 1 x 100 mL | 2 x 100 mL |
| Control Agonist, IL-6 (No. of vials) | 1 | 1 |
| 96-well White, Clear Flat-bottom Tissue Culture-treated Sterile Plates with Lid | 2 | 10 |

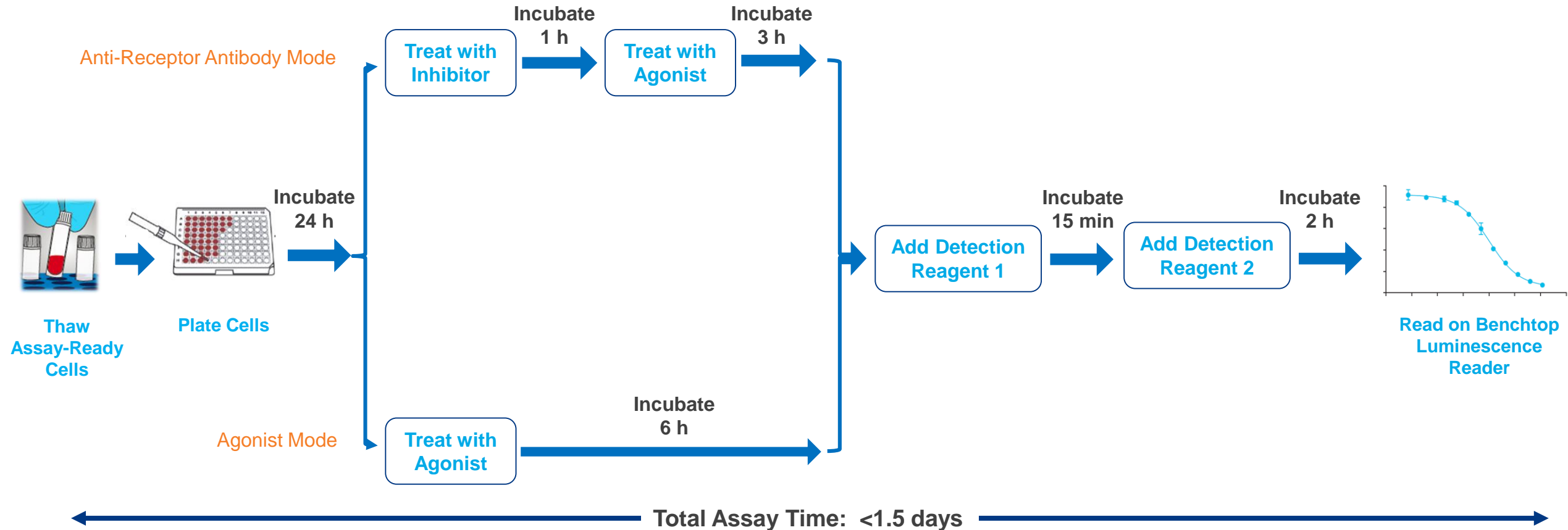
* Cell Plating 5 Reagent is also used for diluting control agonist and tocilizumab in the bioassay.

Sample data

Tocilizumab Bioassay Kit Qualification

Tocilizumab Bioassay Workflow

Simple, Homogenous and Rapid Protocol

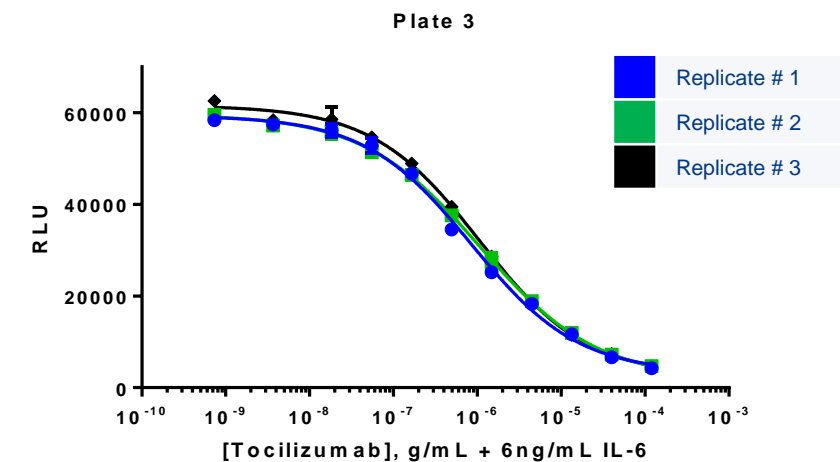
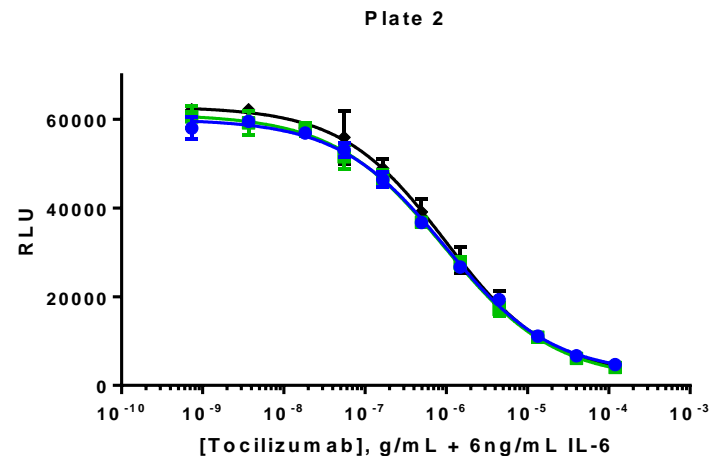
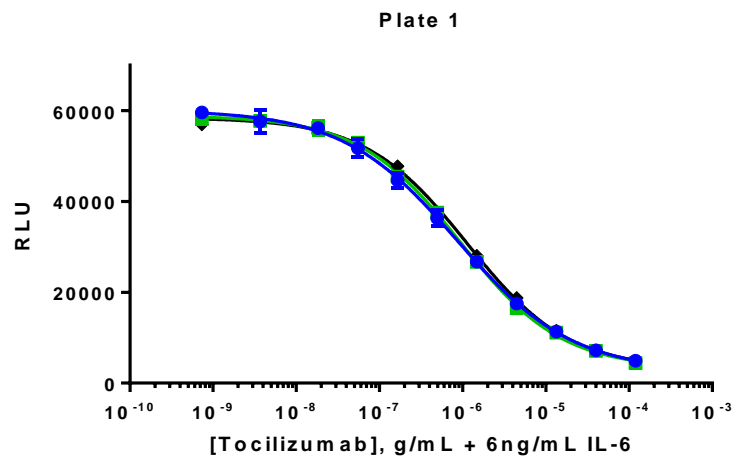


Assay Parameters Assessed

- % CV between eight 11-pt DRCs
- Plate uniformity: EC_{80} and IC_{80} (of drug and stimulus) across entire plate
- Plate-to-Plate variability: 3 plates with 11-pt DRCs run on 3 days
- Slope consistency
- Accuracy, precision and linearity of the assay over a range of 50-150% from two operators
 - Assay developer
 - Assay qualifier
- Parallel line analysis

Tocilizumab Bioassay Qualification with Actemra®

Plate-to-Plate Variability: 3 Plates with Full-plate DRC - Same Day



| Parameter | R1 | R2 | R3 |
|--------------------------|---------|---------|---------|
| S/B | 12.1 | 13.7 | 12.6 |
| Hill Slope | -0.6404 | -0.7106 | -0.7084 |
| IC ₅₀ (ng/mL) | 868 | 935 | 1,131 |

| Parameter | R1 | R2 | R3 |
|--------------------------|---------|---------|---------|
| S/B | 13.2 | 15.2 | 14.1 |
| Hill Slope | -0.6622 | -0.6366 | -0.6781 |
| IC ₅₀ (ng/mL) | 966 | 943 | 972 |

| Parameter | R1 | R2 | R3 |
|--------------------------|--------|--------|---------|
| S/B | 14.5 | 12.8 | 13.8 |
| Hill Slope | -0.683 | -0.624 | -0.6799 |
| IC ₅₀ (ng/mL) | 850 | 1077 | 1063 |

Assay Robustness

Repeatability and Intermediate Precision (Inter-Plate)

| Plate | Sample | S/B | %RSD, S/B | IC ₅₀ , ng/mL | Mean IC ₅₀ , ng/mL | %RSD, IC ₅₀ |
|-------|--------|------|-----------|--------------------------|-------------------------------|------------------------|
| 1 | R1 | 12.1 | 6.4 | 868 | 978 | 13.9 |
| | R2 | 13.7 | | 935 | | |
| | R3 | 12.6 | | 1130 | | |
| 2 | R1 | 13.2 | 7.1 | 966 | 960 | 1.59 |
| | R2 | 15.2 | | 943 | | |
| | R3 | 14.1 | | 972 | | |
| 3 | R1 | 14.5 | 6.2 | 850 | 997 | 12.8 |
| | R2 | 12.8 | | 1080 | | |
| | R3 | 13.8 | | 1060 | | |

Intermediate Precision (Inter-Day)

| Day | IC ₅₀ , ng/mL | Mean IC ₅₀ , ng/mL | %RSD, IC ₅₀ |
|-----|--------------------------|-------------------------------|------------------------|
| 1 | 1250 | 1330 | 24.6 |
| 2 | 1690 | | |
| 3 | 1050 | | |

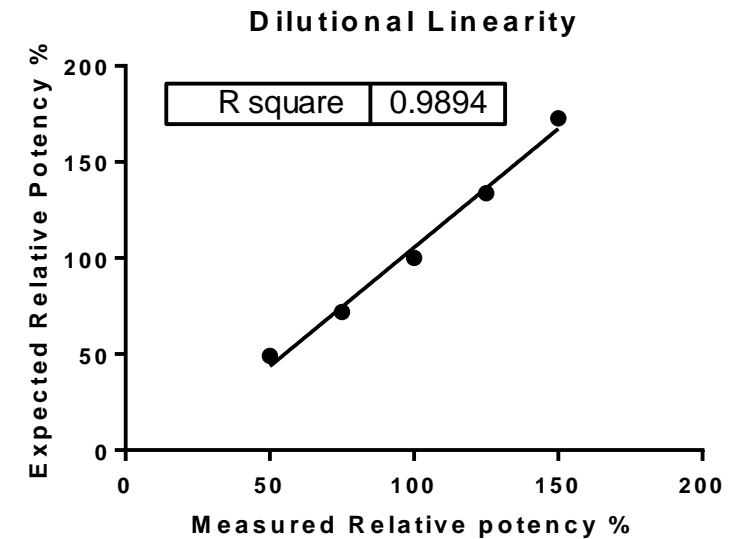
Intermediate Precision (Inter-Plate): 1.89%
Intermediate Precision (Inter-Day): 24.6%

Tocilizumab Bioassay: Qualification with Actemra®

Summary: Accuracy, Precision and Dilutional Linearity (Single Analyst)

| Exp # | Expected RP (%) | Observed RP (%) | Average RP (%) | % RSD | % Recovery |
|-------|-----------------|-----------------|----------------|-------|------------|
| 1 | 150 | 155.5 | 153.3 | 11.2 | 102.2 |
| 2 | | 163.2 | | | |
| 3 | | 141.1 | | | |
| 1 | 125 | 138.6 | 138.2 | 0.7 | 110.6 |
| 2 | | 137.4 | | | |
| 3 | | 138.6 | | | |
| 1 | 75 | 74.1 | 77.5 | 4.8 | 103.3 |
| 2 | | 83.0 | | | |
| 3 | | 75.4 | | | |
| 1 | 50 | 54.8 | 52.9 | 2.1 | 105.8 |
| 2 | | 50.6 | | | |
| 3 | | 53.3 | | | |

Note: RP values calculated in GraphPad Prism

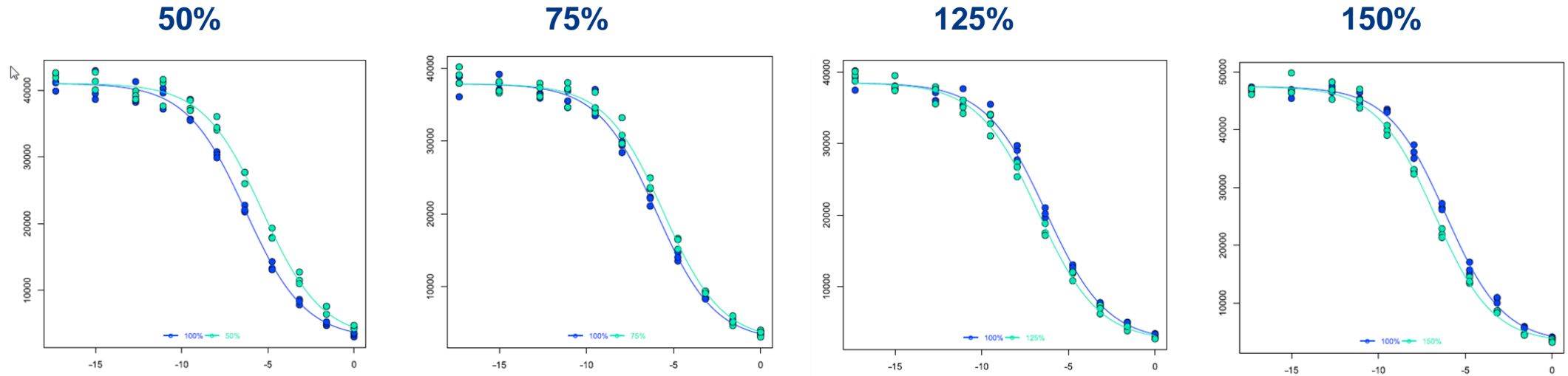


Accuracy: 105.5%
Precision: 4.7%

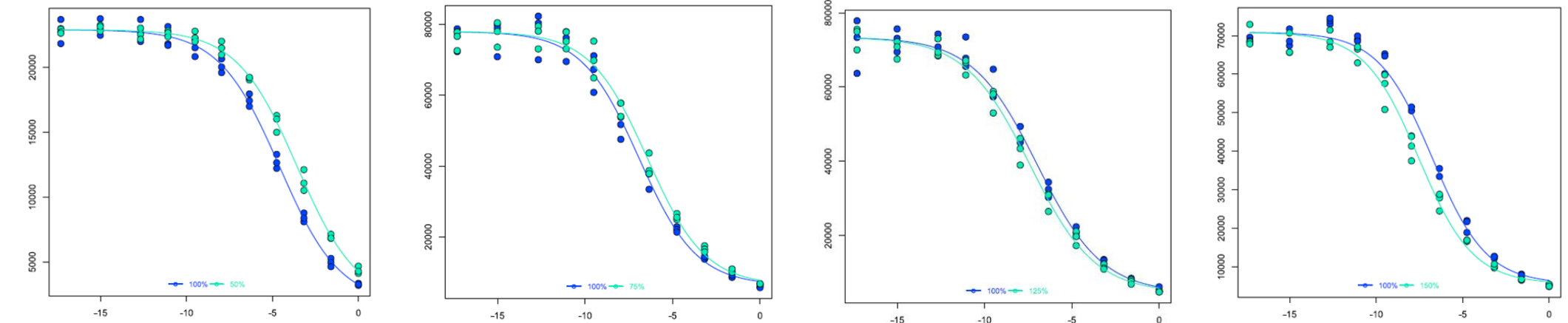
Tocilizumab Bioassay: Qualification with Actemra®

Relative Potency: Parallelism and Potency Estimation (PLA)

Analyst 1



Analyst 2



● Reference
● Sample

Note: RP values calculated in PLA 3.0

Benefits for “Ready-to-Use” Bioassay Kits

Functional response based on drug MOA

Simple protocol; Rapid results

Specific and Sensitive assay

Highly reproducible

Readily Implement in QC with Optimized kit

- Frozen ready-to-assay cells
- Bioassay Detection Reagents
- Cell Plating Reagent
- Dilution Buffer
- Control Agonist
- Tissue Culture-Treated Plates

For More Info, Questions or Technical Support



Web:

[Cell-Based Bioassays for Biologics](#)

Technical Support

For NA:

DRX_SupportUS@eurofinsUS.com

For Europe, Africa & Middle East:

DRX_SupportEurope@eurofinsUS.com

For Asia-Pacific:

AsiaPacificSupport@eurofins.com