# Best Practices for Receptor Occupancy Assays in Clinical Sample Analysis

**Megan Rasmussen, Ph.D., Teresa Urlacher, Ph.D., Johannes Stanta, Ph.D.**

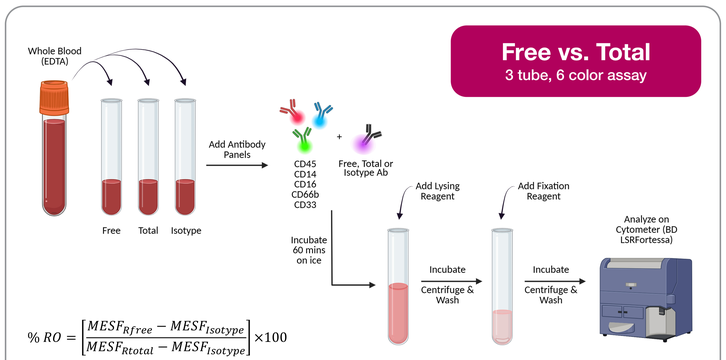
Molecular and Cell Biology, Bioanalytical Services
Celerion, Inc. Lincoln, Nebraska

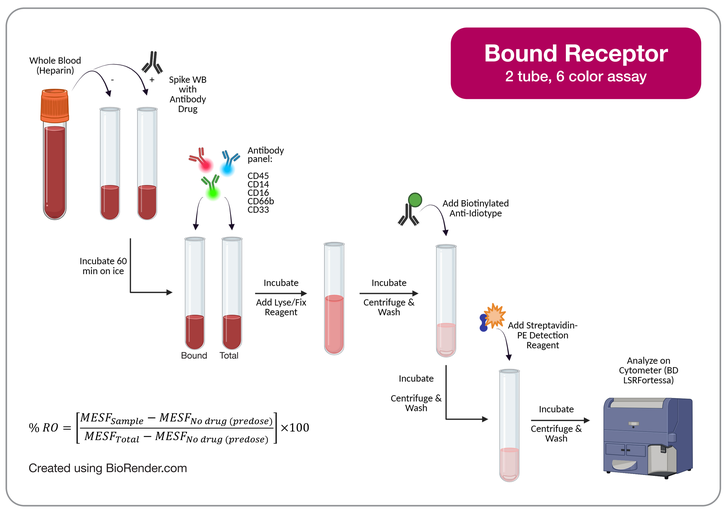
## Background

Flow cytometry-based receptor occupancy (RO) assays are valuable tools for assessing the pharmacodynamic (PD) activity of therapeutic antibodies by quantifying their engagement with cell-surface targets. These assays can measure free, bound, and/or total receptor levels on relevant cell populations, offering mechanistic insight into drug–target interactions.

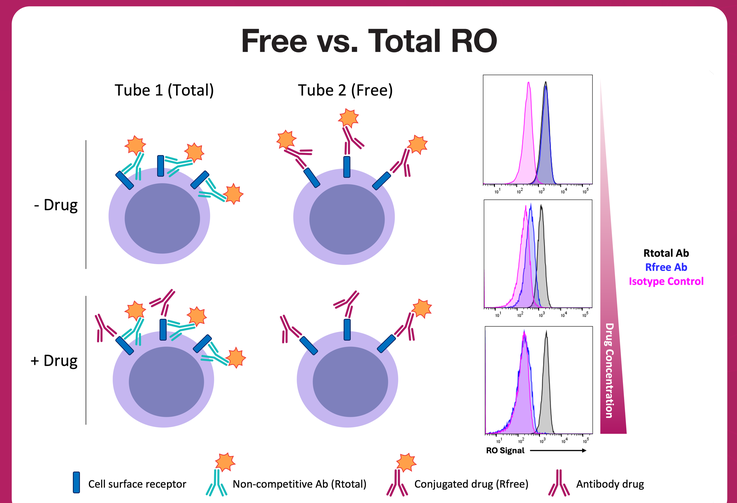
While RO assays are often less complex than high-parameter immunophenotyping panels, they require careful design and optimization. This includes the use of specialized reagents, such as labeled therapeutic antibodies, competing ligands, or anti-idiotype antibodies, and the selection of appropriate gating strategies and controls. RO methods must be customized for the specific therapeutic and biological context and validated as fit-for-purpose to ensure they provide robust, reproducible, and interpretable data across pre-clinical and clinical studies.

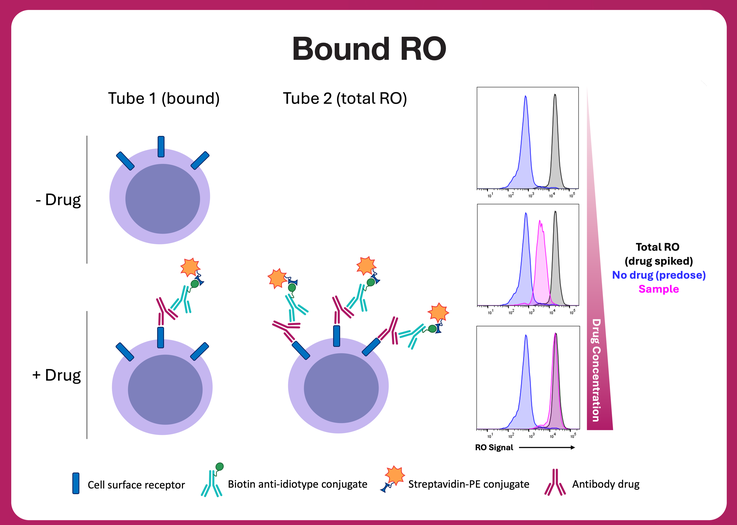
## Methods




# Individualized Approaches for Receptor Occupancy (RO) Assays Provide Critical PD Data in Early-Phase Clinical Trials.



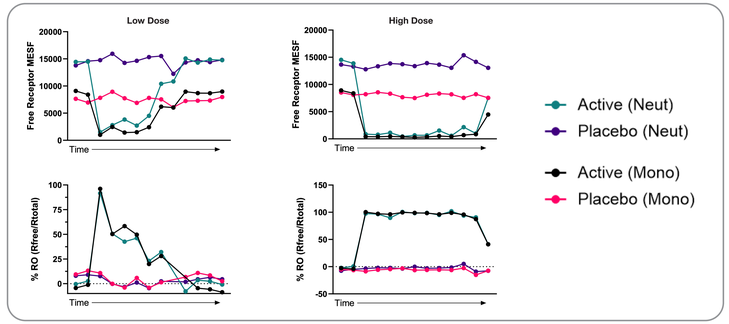




**Each assay was validated following the Type 2 Fit-For-Purpose validation recommendations outlined in the CLSI H62.**

- ☑ Within-Assay Precision
- ☑ Between-Operator Precision
- ☑ Whole Blood Stability
- ☑ Processed Sample Stability
- ☑ Between-Instrument Precision

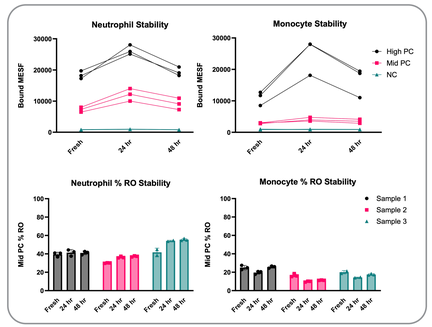
## Results

### Free vs. Total RO




- Co-localized clinic and bioanalytical lab allowed for processing of whole blood within 2 hours of collection
- Samples were analyzed on cytometer within 24 hours of staining
- At highest dose tested, near-complete RO was maintained long-term in neutrophils and monocytes

### Bound RO




| | Condition | Statistic | Neut | Mono |
|---|---|---|---|---|
| Sample 1 | Fresh vs. 24hr | Mean | 40.4 | 22.3 |
| | | %CV | 7.1 | 15.7 |
| | Fresh vs. 48hr | Mean | 40.2 | 25.3 |
| | | %CV | 5.3 | 6.7 |
| Sample 2 | Fresh vs. 24hr | Mean | 33.2 | 13.2 |
| | | %CV | 10.5 | 28.8 |
| | Fresh vs. 48hr | Mean | 33.7 | 13.9 |
| | | %CV | 11.8 | 22.3 |
| Sample 3 | Fresh vs. 24hr | Mean | 47.9 | 17.1 |
| | | %CV | 16.1 | 20.1 |
| | Fresh vs. 48hr | Mean | 50.0 | 18.5 |
| | | %CV | 15.9 | 9.3 |

- Samples were shipped from clinical site to bioanalytical lab and were stained and analyzed upon arrival
- Stability testing of whole blood PCs showed % RO was maintained in neutrophils and monocytes up to 48-hours post-draw with storage at 5°C
- The validated method was successfully used to analyze antibody drug-target engagement in a First-in-Human study conducted over 1 year

## Fit-for-purpose RO assays deliver reproducible PD insights across operators and instruments.

Flow cytometry-based receptor occupancy (RO) assays offer critical pharmacodynamic insights that complement pharmacokinetic data, particularly in early-phase clinical trials where direct evidence of target engagement is often limited. The two case studies, 1) free vs. total and 2) bound receptor assays, demonstrate the necessity of customized assay design, including the selection of appropriate reagents and optimization of workflow parameters. Each assay was run in over 100 independent experiments, emphasizing the importance of achieving consistent sensitivity, precision, and stability.

These findings underscore both the challenges and key considerations in developing and validating robust, fit-for-purpose RO assays to support therapeutic antibody development in clinical settings.

**The authors gratefully acknowledge our pharmaceutical partners who sponsored these studies.**

celerion
Translating Science to Medicine