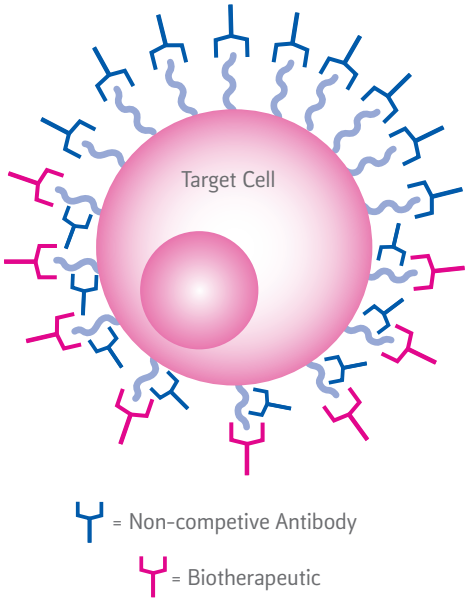
WHITE PAPER

Receptor Occupancy Assay for Assessment of Therapeutic Agents

Receptor occupancy (RO), the measurement of the binding of a therapeutic to its cellular target, is increasingly important in the development of biologically based therapeutic agents. RO assay measurements can be used to determine minimal drug thresholds and guide dosing decisions during the clinical development of a drug. RO assays using flow cytometry describe the qualitative or quantitative measurement of the binding of a therapeutic agent to its cell surface target. Therefore, RO assays can measure the number of cell surface receptors bound by a therapeutic agent or can be designed to address more complicated scenarios such as receptor signaling, internalization or shedding events once a receptor engages a therapeutic agent. Data generated from RO assays can also be used to assess whether doses of an experimental therapeutic agent and its administration schedules lead to predicted levels of receptor occupancy and whether the receptor is modulated (up or down) on cells engaged by the therapeutic agent (1).



There are a variety of approaches that can be used when undertaking RO assays and with the capability to measure distinct subsets in heterogeneous populations, flow cytometry is ideally suited for RO measurements (1-3). These methods utilize fluorescently tagged therapeutic agents or reagents that compete with the biotherapeutic in binding to its target and measures the percentage (%) of unoccupied receptors. In addition, a non-competing reagent can be utilized to measure the total receptor number (total receptors). Based on this principle, RO can be expressed as % free receptors or the relative % of drug-bound receptors. With the emergence of biologic-based therapeutics, the development of RO assays in the field of cancer, autoimmune disease, and other disorders has become an essential component of drug development.

Several factors are critical for developing a clinic-ready receptor occupancy assay

- Reagents for assay development including availability of suitable detection reagents, competing antibodies, non-competing antibodies, etc.
- Controls for monitoring assay performance and for assessing assay specificity and sensitivity
- Matrix selection (malignant cells, PBMC, other blood cells, etc.)
- Clinical sample stability for transport to a specialty central laboratory

A RO assay in peripheral blood samples may utilize any of the cell types, such as lymphocytes, granulocytes, platelets, and RBC, when the target receptor is expressed in these cells. Although a variety of cells may be used for RO assays, we employed peripheral blood as our cell source since the therapeutic target was expressed on both normal circulating blood cells, as well as on its intended malignant target (2). The flow cytometry method for this assay utilized a fluorescently tagged biotherapeutic and an antibody that competed with the biotherapeutic in binding to its target. The total number of receptors irrespective of their interaction with the drug (total receptors) was also determined. Based upon this principle, RO was expressed as the % of drug-bound receptors or %RO.

The following examples (Figures 1 & 2) demonstrate a free receptor type RO assay in lymphocytes and platelets for a biotherapeutic. In this assay, a whole blood sample was spiked with increasing concentrations of the Biotherapeutic A, and then isolated blood cells were incubated with a fixed concentration of competing fluorescently-labeled antibody. The geometric mean fluorescence intensity corresponding to the RO antibody signal was measured (gMFI) in a gated cell sub-population. The gMFI values were then used to calculate % RO. The results demonstrated that the RO increases with increasing concentration of the therapeutic drug.

Figure 1: % RP in PBMC

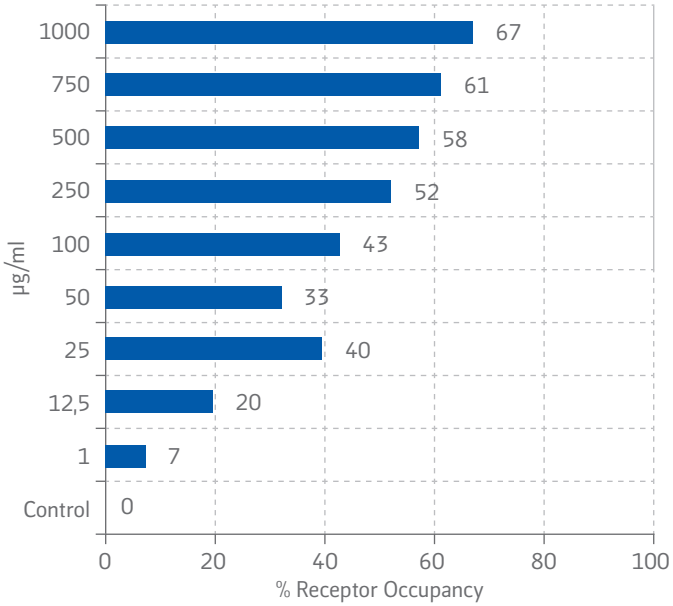
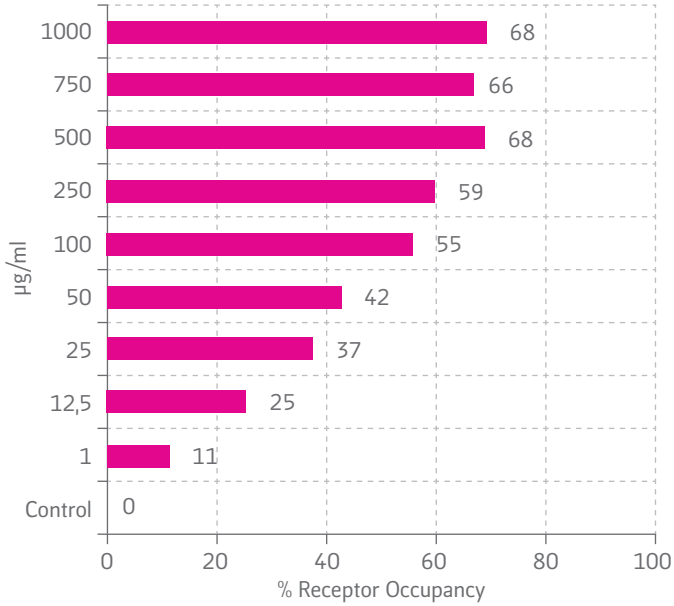
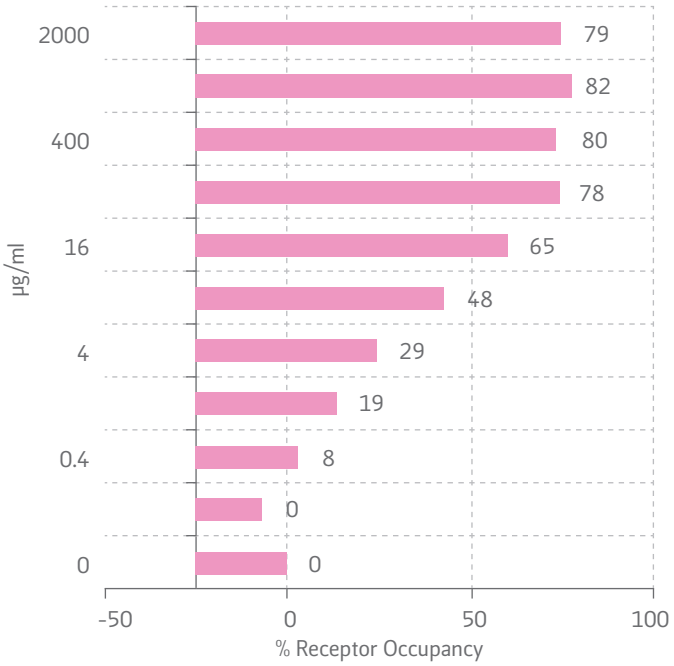


Figure 2: % RP in Platelets



For a different biotherapeutic (Biotherapeutic B), we utilized the platelet as the surrogate for the therapeutic target cell. A fluorescently labeled non-competing antibody was identified and utilized to measure total receptor number and a different fluorescently-labeled competing antibody for the biotherapeutic was used to bind unoccupied receptors. Figure 3 shows the percent receptor occupancy of the biotherapeutic using the equation for the competing (A) and non-competing (B) antibodies as % RO = (100*(1 - (A/B treatment sample)/ (A/B) control or pre-dose sample)). For this assay, median MFI values were used for the calculated values.

Figure 3: % RO in Platelets



MLM Medical Labs develops and validates customized flow cytometry-based RO assays for utilization in preclinical studies and clinical trials. The protocols for blood sample collection, handling, processing and shipping for flow cytometry analyses are tailored to the clinical trial. Learn more about the importance of RO in drug development by visiting www.mlm-labs.com.

References:

1. Stewart, J.J., et al., Role of Receptor Occupancy Assays by Flow Cytometry in Drug Development. Cytometry Part B (Clinical Cytometry), 2016, 90B: 110-116
2. Wyant, T., et al., Development and Validation of Receptor Occupancy Pharmacodynamic Assays Used in the Clinical Development of the Monoclonal Antibody Vedolizumab. Cytometry Part B (Clinical Cytometry), 2016, 90B: 168-1176
3. Green, C. L., et al., Recommendations for the Development and Validation of Flow Cytometry-Based Receptor Occupancy Assays. Cytometry Part B (Clinical Cytometry), 2016, 90B: 141-149.