

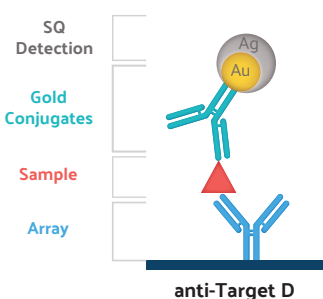
## Arrayed Intuitive Multiplex (AIM)

### Quantitative Immunoassay Platform for Biomarker Detection

Intuitive Biosciences has developed an immunoassay system ready to convert your ELISA or bead-based assay to a more efficient and cost-effective platform. Over the past decade, we have created new assays and transferred existing assays to our platform, both improving efficiencies in workflow and cost savings for our customers. This document describes one type of immunoassay compatible with the AIM platform and provides a comparison to ELISA and bead-based assays for the same targets. The AIM platform can be used for multiplex assays or to detect a single analyte, relying on the highly sensitive SilverQuant<sup>®</sup> chromogenic detection reagents for visible signal.

#### Basic Assay Workflow

- Step 1:** Add diluted sample and incubate.
- Step 2:** Wash and add gold conjugated detection antibodies.
- Step 3:** Wash and add SilverQuant<sup>®</sup> Detection Reagents, incubate for 3 minutes.
- Step 4:** Scan and analyze data.



#### Standard Quantitative Assay

Capture antibodies are arrayed onto proprietary surface. Analytes from sample are bound and visualized with gold-conjugated detection antibodies. A quick enhancement step with the SilverQuant<sup>®</sup> detection reagents results in visible signals.

#### Key Benefits

**Cost and time savings:** Multiplexing means less labor and smaller sample volume requirements. Multiplexing also drastically reduces labor costs.

**Simple protocols:** Like an ELISA protocol, all steps in the assay can be completed with standard lab equipment and user protocols.

**Accommodates matrices of all types:** The AIM platform works with serum, plasma, whole blood, urine, saliva, cell culture supernatants, stool extracts, lavage fluid, blood spot cards, and forensic matrices.

**Multiple assay types:** Quantitative sandwich assays, antigen detection, antibody detection, and competition assays.

**Affordable Analyzer:** All AIM assays are compatible with the AQ series analyzers, a low-cost and rapid scanning system.

### Representative Multiplex Calibration Curve and Sensitivity

*All data were collected with assays run as a multiplex.*

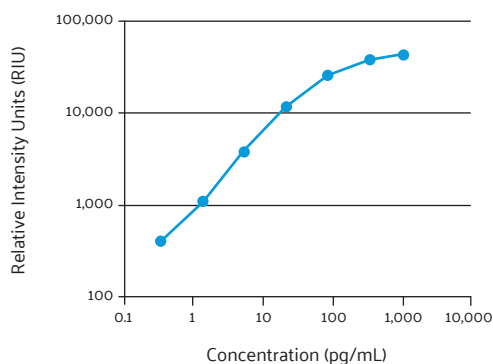
The calibration curves in a quantitative assay are used to calculate analyte concentrations. These were established by using a 4-parameter logistic curve fit model with a  $1/Y^2$  weighting. Concentrations of each analyte were determined from the chromogenic signal measured as Relative Intensity Units (RIU) and back-fit to the calibration curve. The limit of detection (LOD) is a calculated concentration corresponding to the signal 2 standards deviations above the mean of the zero calibrator (background). All assays were performed in multiplex.

The standard curves shown here are for demonstration only. A standard curve must be run each time an assay is run, using the recommended dilution of the standards in the assay buffer.

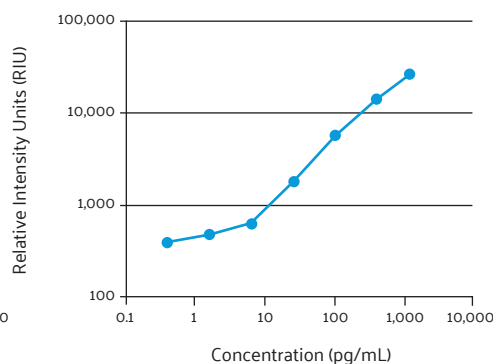
LOD (pg/mL)	Intuitive Bio Multiplex	ELISA	Bead-Based Assay
IL-6	0.25	0.70	1.1
IL-10	2.61	3.9	0.3
TNF-	2.3	5.5	1.5

**Sensitivity.** The limit of detection was determined by adding 2 standard deviations to the mean RIU of 12 replicate measurements of the zero standard. The concentration of each target analyte is shown in pg/mL.

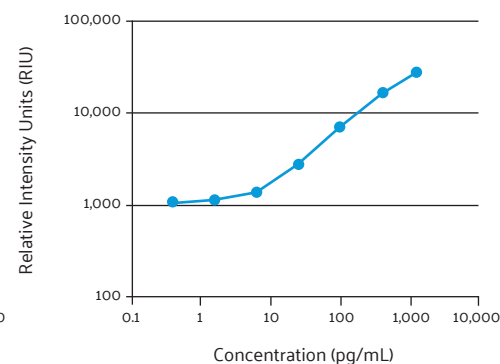
**IL-6**



**IL-10**



**TNF-alpha**



**Multiplex Calibration Curves of the Standard.** The standard curve was created using a dilution series of a multiplex antigen mix. Seven dilutions of the standard mix and a diluent only blank were measured in 12 replicate wells. The mean value for each dilution is plotted for each target.

## Precision

	Average Intra-run %CV	Inter-run %CV
IL-6	2.3	3.0
IL-10	8.6	9.8
TNF-	8.4	8.9
n=	12	36

**Precision of Assay.** Controls were made by spiking the Calibration standard into assay diluent within the quantitative range of the assay. The Average Intra-run is the average percent coefficient of variation (%CV) of the control replicates within an individual run. The Inter-run %CV is the variability of control across multiple runs.

	Control	Average RIU	Measured Conc. (pg/mL)	Intra-run %CV
IL-6	High	32,955	166	2.3
	Mid	25,556	72	1.6
	Low	12,870	19	5.9
IL-10	High	17,914	298	4.4
	Mid	11,566	155	5.4
	Low	5,067	52	14.0
TNF-	High	13,259	253	7.3
	Mid	8,196	112	5.4
	Low	3,700	32	9.7

**Tested Samples.** A standardized sample diluted in serum was run at High, Mid, and Low concentrations. Target antigen concentrations were calculated against the standard curve. Intra-run %CV was calculated for 6 replicate measurements.