

Arrayed Intuitive Multiplex (AIM)

Quantitative Immunoassay Platform for Rapid Biomarker Detection

Intuitive Biosciences has developed an immunoassay system ready to convert any ELISA or bead-based assay to a more efficient and cost-effective platform. The AIM (Arrayed Intuitive Multiplex) platform is uniquely flexible allowing for assay timing from 40 minutes up to 3 hours to run a quantitative multiplex assay. Assay timing is determined based on customer needs and overall design of the assay. This technical brief describes development of a rapid AIM multiplex assay, resulting in protocol that takes a fraction of the time of a conventional ELISA or bead-based assay. The data below demonstrates a comparable performance between the standard format and the rapid AIM assay.

Reduction of Assay Timing

To reduce the incubation times for a standard immunoassay, several steps can be modified to increase the binding kinetics of the antibody-antigen complexes. For the standard assay, all steps were performed at room temperature (24°C). For the rapid assay, the AIM plates were incubated on a heater/shaker for consistent heating and agitation. Table 1 below shows the comparison of the assay timing for each step in the standard and rapid assay. Both assays use the same materials, with some adjustment for concentration of detection reagents in the rapid assay.

AIM = 3 hours 30 minutes	Rapid AIM = 39 minutes
Dilute samples incubate 1 hour	Dilute samples incubate 10 minutes
Wash and incubate with anti-target biotin 1 hour	Wash and incubate with anti-target biotin 5 minutes
Wash and incubate with anti-biotin gold 1 hour	Wash and incubate with anti-biotin gold 5 minutes
Wash and develop with SilverQuant 3 minutes	Wash and develop with SilverQuant 3 minutes
Scan and analyze 5 minutes	Scan and analyze 5 minutes

Table 1. The assay steps and timing are shown for the standard AIM Assay on the left, while the Rapid AIM assays steps are shown on the right. Assay timing shown above in each is the total time including the washing steps, scanning, and analysis.

Representative Multiplex Calibration Curve and Sensitivity

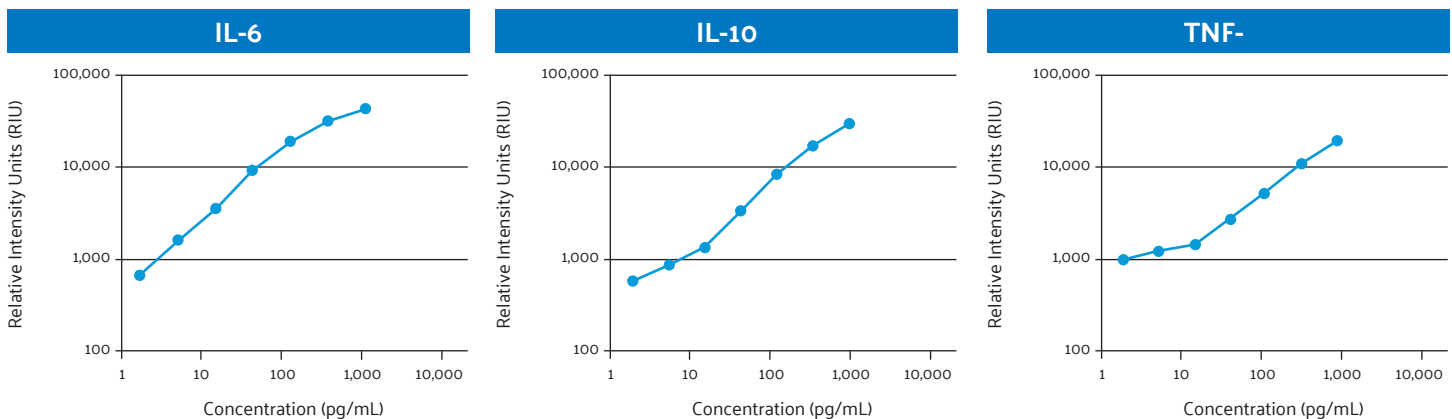
All data were collected with assays run as a multiplex.

For the comparison of the standard and rapid AIM assay formats, a serial dilution of a recombinant antigen standard was used to determine the limit of detection for each protocol. Twelve replicate measurements of each concentration of the standard were used for each condition. Calibration curves were used to calculate measurable analyte concentrations, 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 standard deviations above the mean of the zero calibrator (background). All assays were performed in multiplex.

LOD (pg/mL)	Standard AIM	Rapid AIM
IL-6	0.25	0.96
IL-10	2.61	5.57
TNF-	2.3	8.59

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.

When comparing the sensitivity of the two assay methods, the LOD for the standard assay format is lower than the rapid assay. When selecting an assay format, consideration should be given to the biological significance of the lower limits of detection in each assay format. The rapid assay can provide fast and reliable results in a much shorter time frame. Individual standard curves are shown below for the rapid AIM assay performed in multiplex.



Multiplex Calibration Curves of the Standard for the Rapid Assay. The standard curve was created using a dilution series of a multiplex antigen mix and assayed using the rapid assay format. 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.

Summary

The AIM rapid assay format provides a fast and accurate test result.

The AIM multiplex platform is a flexible multiplex immunoassay system. The standard assay formats provide high sensitivity within 3.5 hours. The rapid assay can reduce the time to less than 40 minutes, while still obtaining accurate and reproducible results. Not all targets will be compatible with a rapid assay format, so consideration should be given to the laboratory requirements when selecting an assay protocol to perform.