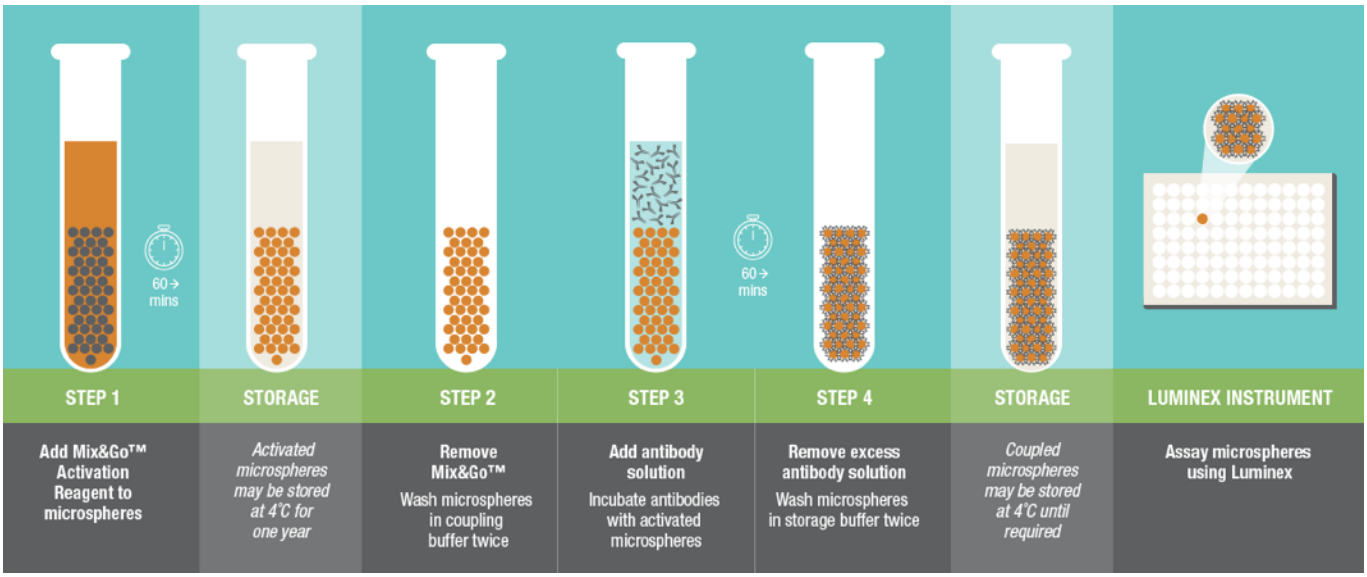
Simple Conversion from ELISA to Luminex® Using the AMG™ Activation Kit

Summary

Luminex® MagPlex® Microspheres may be coupled with antibodies specified for use in ELISA using Anteo Technologies' AMG™ Activation Kit. This kit uses Mix&Go™ technology to strongly couple antibodies to multiplex microspheres such as Luminex® MagPlex® Microspheres.

This application note gives the example of coupling two anti-cytokine antibodies to MagPlex Microspheres. A Multiplex immunoassay was then performed using the Luminex® system with the matched detection antibodies and standards.

The Luminex system uses fluorescence detection allowing for much greater sensitivity and dynamic range when compared with colourimetric ELISA methods. The system also affords the option of multiplexing multiple analytes in a single well. Typically, 100 microspheres are read per analyte per well, providing many replicates and high quality data sets. This greatly reduces the number of wells required for the immunoassay. The amount of sample required, the run time and time taken to read the assay are also reduced. Performing the same assay in an ELISA format often requires significant assay optimisation in the first instance. Also required are higher quantities of sample, more hands on assay time and reagents. In this example, two analytes were run simultaneously in a single well as detailed below.



Materials and Methods

Activation and Coupling

AMG™ Activation Kit for Multiplex Microspheres
(Anteo Technologies A-LMPAKMM-10)
- Mix&Go Activation Reagent A
- Coupling Buffer, Formulation B
- Storage Buffer, Formulation B

Luminex® MagPlex® Microspheres
(Luminex Corporation MC10XXX-01)
IL-6 Capture Antibody (BD Pharmigen™ 554543)
TNF Capture Antibody (BD Pharmigen™ 551220)

Luminex microspheres were resuspended by gentle inversion for 1-2 minutes, as per the manufacturer's included product information sheet. 100 µL of this suspension was then transferred to a 1.5 mL microcentrifuge tube. The tube was placed on a magnetic separator for at least 1 minute until the supernatant was clear. The supernatant was then aspirated and the tube removed from the magnetic separator. 100 µL of the Mix&Go Activation Reagent was then added to the microsphere pellet and resuspended using a vortex mixer on medium speed. The tube was then placed onto a tube rotator and incubated at room temperature (20˚C – 25˚C) for 1 hour. Activated microspheres may be stored for future use for up to 12 months at 4˚C. This is unique to the AMG Activation Kit and is not possible with S-NHS/EDC coupling chemistries as the active esters hydrolyse in water over time.

The microspheres were washed twice in coupling buffer before coupling with antibody. In a separate tube the antibodies were diluted to 25 µg/mL in coupling buffer. 100 µL of the antibody solution was then added to the washed microsphere pellet. The tube was subsequently placed onto a tube rotator and incubated at room temperature (20˚C – 25˚C) for 1 hour.

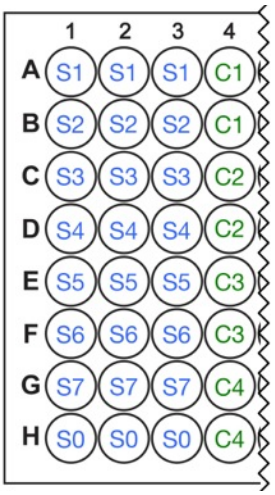
The microspheres were then washed with storage buffer three times and stored at 4˚C. The antibody-coupled microspheres should be assessed for individual use and storage stability conditions, as this can vary depending on the proteins used.

Multiplex Assay

IL-6 Standard (BD Pharmigen™ 550071)
TNF Standard (BD Pharmigen™ 554618)
IL-6 Detection Antibody (BD Pharmigen™ 554546)
TNF Detection Antibody (BD Pharmigen™ 554511)
Streptavidin-RPE (Prozyme® PJRS14)
Assay Buffer (PBS 1% BSA 0.05% TWEEN® 20)
Wash Buffer (PBS 0.05% TWEEN® 20)

Into a single tube, the microspheres were diluted by a factor of 200, using assay buffer. The Standards and Controls were prepared in tubes by serial dilution of the antigen. Standards by a factor of three and Controls by a factor of five (Figure 1).

To a 96 well filter plate, 25 µL of the diluted microsphere mixture was added to the first 4 columns. After the solution was removed, 25 µL of the standards and controls were added as per Figure 1. This was then incubated for 60 minutes at room temperature on a plate shaker.



Standards		Controls	
S1	1,800 pg/mL	C1	1,000 pg/mL
S2	600 pg/mL	C2	200 pg/mL
S3	200 pg/mL	C3	40 pg/mL
S4	66.67 pg/mL	C4	8 pg/mL
S5	22.22 pg/mL		
S6	7.4 pg/mL		
S7	2.47 pg/mL		
S0	0 pg/mL		

Figure 1. Plate Layout and corresponding dilution table.

Detection antibody was prepared by diluting the detection antibodies to 1 µg/mL (final concentration) in a single tube. To the wells containing microspheres, 25 µL of the detection antibody mixture was added and incubated for a further 30 minutes.

25 µL of the streptavidin RPE solution, diluted to 20 µg/mL, was added to the wells containing microspheres and incubated for 15 minutes.

The plate was then washed 3 times with wash buffer and read on a Luminex® 100 system using the bead region corresponding to the microspheres used in the assay. The median fluorescent intensity was collected for each well.

Results

The Bio-Plex® Manager software was used to generate the standard curve. The values for each standard were entered and analysed using a 5-place regression model. From this data the software calculated the controls which where then plotted against the expected values in Microsoft Excel. The results for the ELISA method are shown by the standard curves in Figure 4 and 5. An increase in dynamic range and lower limits of detection were observed with the Luminex multiplexing method as shown in Figure 2 and 3. A plot of the actual observed results versus the expected values for both analytes tested resulted in a slope with an R^2 value > 0.999 as shown in Figure 6 and 7.

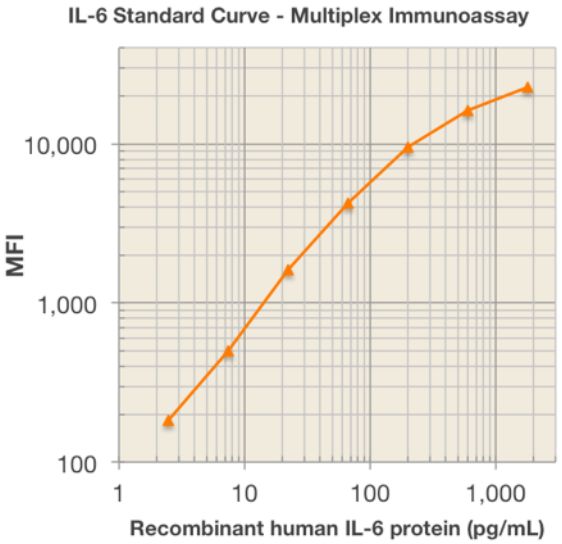


Figure 2. IL-6 Standard curve generated using the AMG Activation kit for Multiplex Microspheres

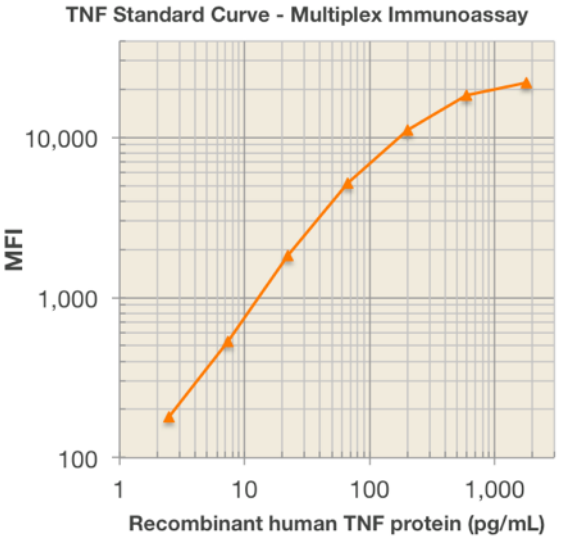


Figure 3. TNF Standard curve generated using the AMG Activation kit for Multiplex Microspheres.

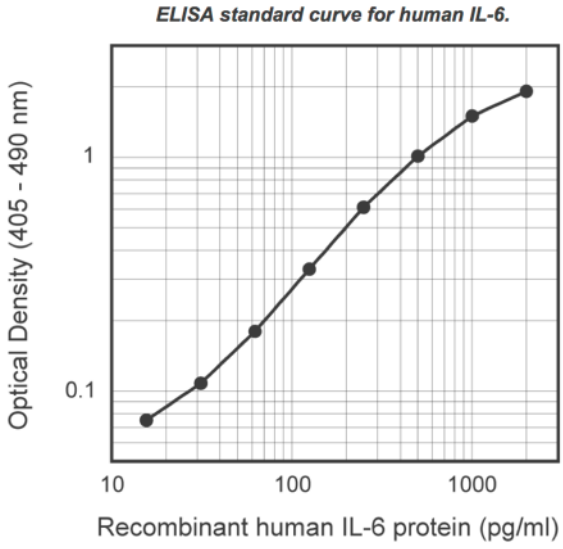


Figure 4. IL-6 Standard curve taken from the BD Biosciences Technical Data Sheet: Purified Rat Anti-Human IL-6 (554543 Rev. 1).

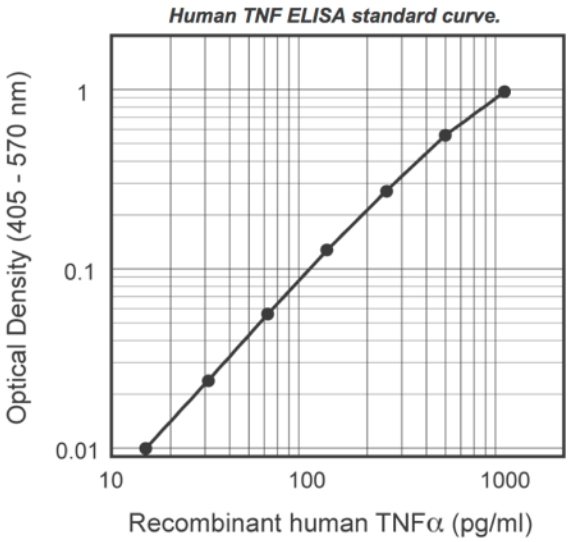


Figure 5: TNF Standard curve taken from the BD Biosciences Technical Data Sheet: Purified Mouse Anti-Human TNF (551220 Rev. 1).

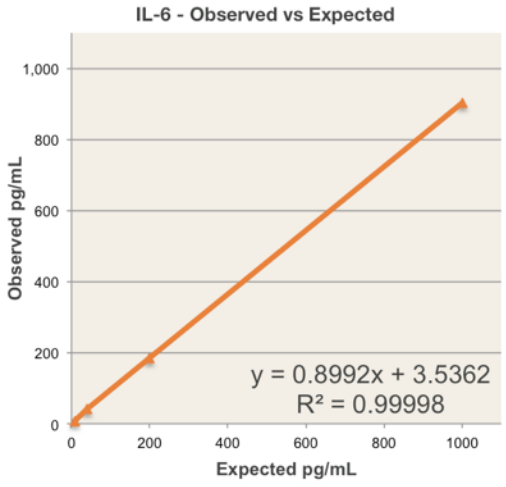


Figure 6: Observed vs Expected for the four Controls of IL-6.

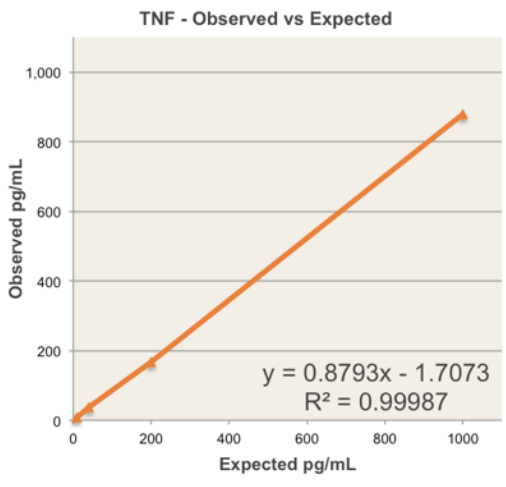


Figure 7: Observed vs Expected for the four Controls of TNF.

Discussion

The AMG™ Activation Kit from Anteo Technologies, is simple and easy to use. It enables researchers to easily develop multiplex immunoassays on the Luminex® system using the same antibodies typically used for ELISA. It removes the need to specifically test for new antibody pairs that are compatible with the alternative approach of antibody coupling onto Luminex microspheres that relies on covalent EDC/NHS chemistry.

The data shows a multiplex cytokine immunoassay developed using antibodies from BD Biosciences specified for ELISA use. The standard curves generated compare well to the standard curves shown in the BD technical data sheets. The curves generated on the Luminex system show that the limit of detection of the assay can be further improved, showing the greater sensitivity possible on this system. High precision and accurate results are obtained when solving Controls using the standard curve. In this example, the expected vs obtained slope was almost a perfect correlation, with an R^2 value > 0.999. The AMG Activation Kit can be used to effortlessly transfer ELISA methods onto the Luminex multiplex platform without the need for extensive optimisation.

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