Development of an IL-6 Assay on MagPlex®-Avidin Microspheres using Rapid IgG Biotinylation and RPE-IgG labeling kits

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Abstract

- An IL-6 sandwich immunoassay was developed on MagPlex-Avidin Microspheres using a biotinylated capture antibody and a Phycoerythrin-labeled detection antibody.
- Both the capture and detection antibodies were affinity-purified goat polyclonal IL-6.
- The capture antibody was biotinylated using the MossLink-Biotin Conjugation kit, desalted, and bound to MagPlex-Avidin microspheres.
- The detection antibody was directly labeled with R-Phycoerythrin using the MossLink-RPE Conjugation Kit. The RPE-labeled detection antibody was characterized by HPLC and was used in the assay without further purification.
- The antibody-coated microspheres were incubated with recombinant IL-6 for one hour, washed with 1XPBST, and then incubated with RPE-labeled detection antibody for one hour.
- After washing, the fluorescent signal was then measured on the Luminex 100.
- The results were compared with a similar assay in which the capture antibody was covalently bound to xMAP® beads and IL-6 was detected with biotinylated anti-IL-6 followed by streptavidin-phycoerythrin.
- Results showed that the immunoassay detected recombinant human IL-6 with a linear dose-response and excellent sensitivity.
- The combination of rapid IgG labeling methods from Moss and Luminex xMAP® technology allows researchers to rapidly develop and validate new assays in a short time frame with reproducible reagents and results.

Background

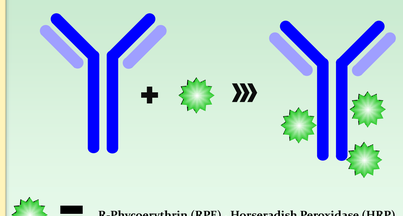
- MagPlex®-Avidin microspheres are spectrally distinct avidin-coupled paramagnetic microparticles that have found wide application for their ease of use in multiplexed Luminex assays.
- When reacted with a biotinylated antibody, the beads can be used to capture a specific target antigen as part of a "sandwich" immunoassay.
- Whereas assays developed with antibody covalently bound to microparticles most often use biotinylated detection antibodies, biotinylated detection antibodies cannot be used in assays using avidin-coupled beads due to the chance that they might bind directly to the avidin coated beads. In addition, streptavidin might bind to an unbound biotin on the capture antibody.
- For assays based on avidin-coupled beads, the detection antibodies must be directly labeled with a fluor such as R-phycoerythrin (RPE). Conjugation of small chemical fluors to antibodies is relatively easy, but these fluors generally do not give sufficient sensitivity in most Luminex assays. However, conjugation of IgG to a large fluorescent protein such as RPE is typically much more difficult.
- We have developed rapid and reproducible kits for labeling IgG with RPE and biotin that are remarkably easy, reproducible, and provide enhanced stability of the resulting conjugates.

Materials/Methods

- Biotinylated and non-biotinylated goat polyclonal anti-human IL-6 and recombinant IL-6 standard was purchased from Peprotech.
- Microplex® and MagPlex®-Avidin beads were obtained from Luminex Corp.
- Assay Buffer was 1X PBST containing 10 mg/ml BSA
- Wash Buffer was 1X PBST (1X PBS containing 0.5% Tween-20
- All analyses were performed on a Luminex 100® calibrated at the high reporter gain setting (high PMT) setting using LDS 1.7 software.
- All reactions take place and beads are washed in Pall AcroPrep Advance Filter Plates for Multiplexing using a Pall vacuum filtration system.
- Polyclonal Goat anti-human IL-6 was labeled with R-Phycoerythrin using the MossLink-RPE kit.
- Polyclonal Goat anti-human IL-6 was labeled with biotin using the MossLink-Biotin kit.

Antibody Labeling

MossLink Conjugation Kits



R-Phycoerythrin (RPE) , Horseradish Peroxidase (HRP) or Biotin

Biotin Labeling using the MossLink-Biotin Kit

100 µg of polyclonal Goat-anti human IL6 was reconstituted in 100 µL of 1X PBS

The IgG solution was added to a tube containing a pre-aliquoted amount of biotin labeling reagent.

The vial was vortexed for 10 seconds and then placed on a shaker for one hour.

The labeled antibody was desalted into 1X PBS using Pierce Zeba desalting columns.

The antibody was stored at 2-8º before use.

RPE Labeling using the MossLink-RPE Kit

100 µg of polyclonal Goat-anti human IL6 was reconstituted in 100 µL of 1X PBS

Activation solution was prepared and added to the IgG and incubated for one hour at room temperature.

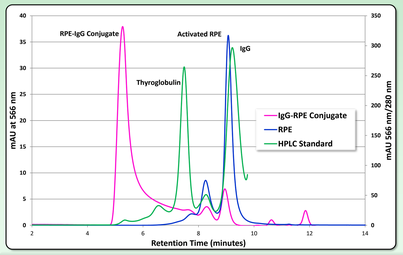
The activated antibody was desalted into 1X PBS using spin desalting columns.

8 µl of a 20 mg/ml solution of pre-activated R-Phycoerythrin was added to the activated IgG and the mixture was incubated overnight at RT.

The vial was vortexed for 10 seconds and then placed on a shaker for one hour.

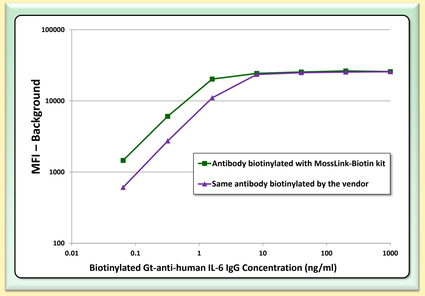
The labeled antibody was desalted into 1X PBS using Pierce Zeba desalting columns.

Characterization of the RPE-IgG Conjugate by HPLC



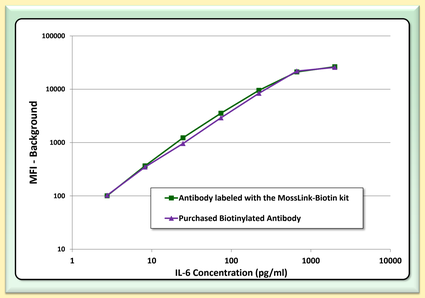
Luminex Assay I: Comparison of Biotinylated anti-IL-6 Antibody on MagPlex®-Avidin Beads

- A serial dilution of biotinylated goat-anti-human IL-6 was added to Mag-Avidin beads and incubated at room temperature for one hour.
- The beads were washed 3 times with 200 µL 1X PBS.
- RPE-labeled Donkey-anti-goat IgG Conjugate (4 ug/ml) was added to the beads and incubated with mixing for one hour at room temperature
- The beads were washed 3X with 200 µL 1X PBST.
- Beasd were resuspended in 100 µL of 1X PBST.
- MFI was measured on the Luminex 100®.

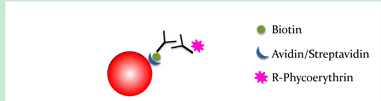


Luminex Assay II: IL-6 Sandwich Assay with MicroPlex® Beads and Biotinylated IL-6 Detection Antibody

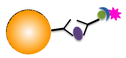
- Microplex® beads had previously been coupled with polyclonal Goat-anti-human IL-6 using standard EDC methods.
- Recombinant IL-6 was captured onto the beads for one hour.
- Beads were washed 3X with 1X PBST
- Bound antibody was detected with a biotin-labeled Goat anti-human antibody for 60 minutes followed by Moss streptavidin-phycoerythrin (SAPE) for 30 minutes
- Beads were washed 3X with 1X PBST.
- MFI was measured on the Luminex 100®.



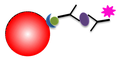
Assay Formats



Assay I:
MagPlex-Avidin® Beads capture biotinylated Goat anti-IL-6 IgG. The bound IgG is detected with a donkey-anti-goat PE conjugate



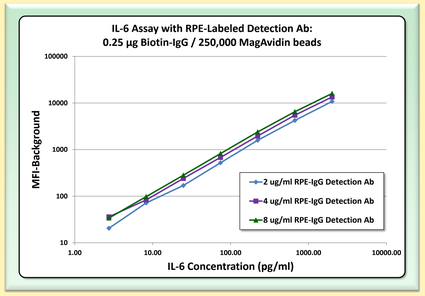
Assay II:
MicroPlex® Beads were covalently coupled to goat anti-IL-6 IgG. Recombinant IL-6 standard is captured and subsquently detected with biotinylated anti-IL-6 and then streptavidin phycoerythrin (SAPE)

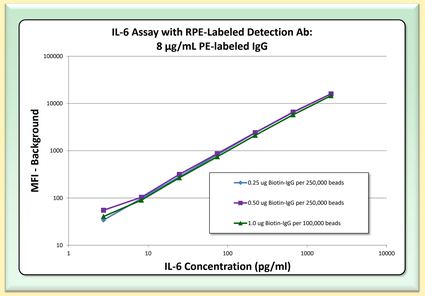


Assay III:
Biotinylated goat-anti-IL-6 is captured onto MagPlex-Avidin® beads. Recombinant IL-6 standard is captured and subsquently detected with phycoerythrin-labeled anti-IL-6.

Luminex Assay III: IL-6 Sandwich Assay with MagPlex®-Avidin Beads and IL-6-RPE Conjugate

- Biotinylated anti-IL-6 antibody was bound to MagPlex®-Avidin beads at three different levels for one hour at room temperture.
- The beads were washed four times with 1XPBST
- Recombinant IL-6 was captured onto the beads for one hour.
- Beads were washed 3X with 1X PBST
- Captured, recombinant IL-6 was detected with an anti-IL-6 antibody directly labeled with RPE at 3 concentrations for 30 minutes.
- Beads were washed 3X with 1X PBST and resususpended in 100 µl 1XPBST.
- MFI was measured on the Luminex 100®.





Conclusions

- Anti-human IL-6 was rapidly biotinylated with the MossLink-Biotin Labeling kit and bound more efficiently to MagPlex-Avidin® beads than the same antibody that had been biotinylated by the vendor.
- Avidin-binding data suggest that the antibody labeled with the MossLink-Biotin Kit is labeled with more biotin moieties than the commercial biotinylated antibody.
- In an IL-6 sandwich assay using covalently coupled MicroPlex ® beads, the biotinylated anti-IL-6 antibody performed slightly better than the same antibody biotinylated by the vendor. The higher level of biotinylation on the Moss antibody did not interfere with the binding of the antibody to the target (recombinant IL-6).
- The biotinylated anti-IL-6 antibody was successfully bound to MagPlex-Avidin beads that were then used in an IL-6 sandwich immunoassay . As little as 0.25 µg of biotinylated antibody could be used to prepare 250,000 coupled beads (100 tests at 2500 beads/test).
- Anti-human IL-6 antibody was conjugated to R-Phycoerythrin using the MossLink-RPE kit and used to directly detect IL-6 on antibody-bound MagPlex-Avidin beads. At least 4 µg/ml of RPE conjugate should be used, although slightly better results can be obtained by using the conjugate at 8 ug/ml.
- In both sandwich immunoassay formats, the 3.2 pg/ml IL-6 standard was easily detectable. The format with covalently coupled beads and a biotinylated detection antibody gave higher overall signals, likely due to the amplification effect of using the biotinylated detection antibody followed by streptavidin-phycoerythrin.
- MagPlex-Avidin® beads in conjunction with a biotinylated capture antibody and a directly labeled PE conjugate offer a very efficient way to test many antibodies and assay configurations and provides an assay protocol with fewer steps.

Additional Info

For additional information regarding experimental details, labeling protocols, conjugation kits, or custom conjugates for Luminex assays please contact :

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