A Single Coupling Approach To Bind Antibodies Of Diverse Species And Classes

Introduction

Antibody coupling plays a critical role in the life sciences. The ability to bind a range of different types of antibodies functionally onto magnetic particles using a single chemistry frees scientists' time for further investigative studies. There are many applications possible using an antibody coupled particle such as lateral flow, immunoprecipitation, purification or depletion of biomolecules and pull-down assays. Scientists are often required to test different chemistries or methodologies to identify the one best suited to a given antibody.

Passive adsorption is an example of a possible method of antibody attachment. This utilises hydrophobic interactions to facilitate binding onto the hydrophobic solid support surface such as polystyrene. As a result, there is no control of the orientation of the antibody resulting in reduced overall functionality. Covalent chemistries offer another approach for coupling antibody that involve modification of the surface to target the amino groups of the antibody. However if excess unreacted reagent is not removed during the conjugation, the carboxyl groups of the antibody can become activated [1] leading to antibody crosslinking instead of antibody-particle coupling. Anteo offers an enabling technology that combines the ease of handling of passive adsorption with the strength of binding of covalent chemistry.

Anteo's surface coating technology allows the user to bind a range of different antibody types using the Antibody Coupling Kit. The kit contains all of the reagents and buffers necessary to allow the user to couple their required antibody simply and easily.

Summary

Traditional covalent chemistries use harsh chemicals and require expertise in the techniques of diverse covalent coupling methodologies. The Antibody Coupling Kit was made to address issues such as: difficulties in binding certain antibodies with traditional covalent chemistries, antibody wastage, and incorrect antibody orientation. Anteo's technology offers scientists the flexibility to bind any antibody onto a solid support surface through the use of polymeric metal complexes. The polymeric metal nature of the technology allows multi-valent binding of the target antibody through chelation to the electron donating groups located in the Fc region of the antibody. Anteo's kit promotes gentle monolayer binding, meaning proteins assemble in the correct orientation while reducing the amount of damaged proteins, leading to increased functionality of antibodies and less antibodies used for the experiment.

This application note demonstrates the ability of the Antibody Coupling Kit to bind Mouse IgG, Mouse IgM, Rabbit IgG, Human IgG and Human IgM antibodies onto 200 nm magnetic particles using a particle-based fluorescent antibody loading assay.

Materials And Methods

Antibody Coupling Kit (Cat # A-VMPABMP-50):
- Particle Pretreatment Solution
- Particle Activation Solution
- Particle Intermediate Solution
- Coupling Buffer
- Blocking Buffer
- Storage Buffer

- AllMag SuperParamagnetic Nanoparticles (Allrun, Cat # PM3-020)
- Assay Buffer: 1% BSA in 10mM PBS + 0.05% Tween 20 pH 7.4
- Wash Buffer: 10mM PBS + 0.05% Tween 20 pH 7.4
- Plate: 96-well white polypropylene round bottom plate (Greiner, Cat #: 650207)
- Plate magnet: BioMag® 96-Well Plate Separator (Polysciences Inc., cat #: 8MB4109S-1)
- Readout: Tecan Infinite M200 Pro (Tecan)

Table 1: Antibodies used for coupling and their respective detection reagent

Antibody For Coupling	Detection Reagent
Mouse IgM (Lampire, Cat # 7404317)	R-Phycoerythrin AffiniPure F(ab')$_2$ Fragment Goat Anti-Mouse IgM, μ Chain Specific (Jackson, Cat # 115-116-075)
Mouse IgG (Lampire, Cat # 7404304)	R-Phycoerythrin AffiniPure F(ab')$_2$ Fragment Goat Anti-Mouse IgG, F(ab')$_2$ Fragment Specific (Jackson, Cat # 115-116-072)
Rabbit IgG (Lampire, Cat # 7406404)	R-Phycoerythrin AffiniPure F(ab')$_2$ Fragment Goat Anti-Rabbit IgG (H+L) (Jackson, Cat # 111-116-144)
Human IgG (Lampire, Cat # 7403704)	R-Phycoerythrin AffiniPure F(ab')$_2$ Fragment Goat Anti-Human IgG (H+L) (Jackson, Cat # 109-116-088)

Magnetic Particle Activation And Coupling
Magnetic particle activation and coupling were prepared as per the Antibody Coupling Kit instructions and detailed below.

Preparation For Particle Activation
The magnetic particles were resuspended by vortex and sonication. An aliquot of 1 mg of the magnetic particles was taken and then placed on a magnet to remove the supernatant. The magnetic particles were resuspended in Particle Pretreatment Solution, with a vortex of the tube to ensure that the contents were dispersed.

Particle Activation
To a fresh tube, 90% of the final required volume for the antibody coupling was added with Particle Activation Solution. The magnetic particles were then added to the tube containing the Particle Activation solution. The magnetic particles were resuspended with a vortex, this was then followed by sonication of the tube to ensure that the contents were dispersed prior to incubation at room temperature. After incubation of the magnetic particles, the Particle Activation Solution was removed and replaced with Particle Intermediate Solution.

Antibody Coupling And Blocking

The supernatant from the activated magnetic particles was removed. The magnetic particles were then resuspended with Coupling Buffer, with a vortex of the tube to ensure that the contents were dispersed.

These steps were repeated twice, resulting in a total of three washes. In a new tube, the required antibody was prepared to ensure that the final concentration would be at 50 µg of antibody /mg beads. The magnetic particles were then added to the tube containing the antibody. The magnetic particles were resuspended with a vortex, this was then followed by sonication of the tube to ensure that the contents were dispersed prior to incubation at room temperature.

Following the incubation period, blocking buffer was added to the magnetic particle solution. The magnetic particles were then vortexed and allowed to incubate. After the incubation period, the supernatant of the magnetic particles was removed and replaced with Storage Buffer. The magnetic particles were washed again before resuspending at the starting particle concentration.

Assessment Of Antibody Coupling By Fluorescent Bead-based Assay

The antibody coupled magnetic particles were diluted using 1% BSA in 10mM PBS + 0.05% Tween20 pH 7.4. 50 µL of the antibody coupled magnetic particles were subsequently added to a round-bottomed 96-well plate. The detection reagents (refer to Table 1) were prepared at 10µg/mL using 1% BSA in 10mM PBS + 0.05% Tween20 pH 7.4. 50 µL of the detection reagent was added to the round-bottomed 96-well plate. As a result of the R-Phycoerythrin fluorophore label being light sensitive, the plate was covered from light and was allowed to incubate at room temperature.

Following the incubation period, the plate was placed on a magnet with the supernatant removed. The magnetic particles were then resuspended in 100 µL of 10mM PBS + 0.05% Tween20 pH 7.4. These steps were repeated four times, resulting in a total of five washes. The plate containing the magnetic particles was measured using a Tecan infinite M200 PRO plate reader (refer to Table 2).

Table 2: Tecan infinite M200 PRO plate reader configuration

Variables	Output
Mode	Fluorescence Top Reading
Excitation Wavelength	546 nm
Emission Wavelength	575 nm
Excitation Bandwidth	9 nm
Emission Bandwidth	20 nm
Gain	72

Results

The following figures 1, 2 and 3 show the results obtained through the use of a fluorescent bead-based assay. From the data, binding of the detection reagent to the antibody coupled magnetic particles was achieved. This occurred across all the different species and classes of antibodies tested. The use of assay buffer alone did not show any observable background binding.

Figure 1: Fluorescent antibody loading assay of IgA coupling on magnetic particles. Replicates showing both specific binding output and blank assay background results for the range of specific RPE labeled detection reagents.

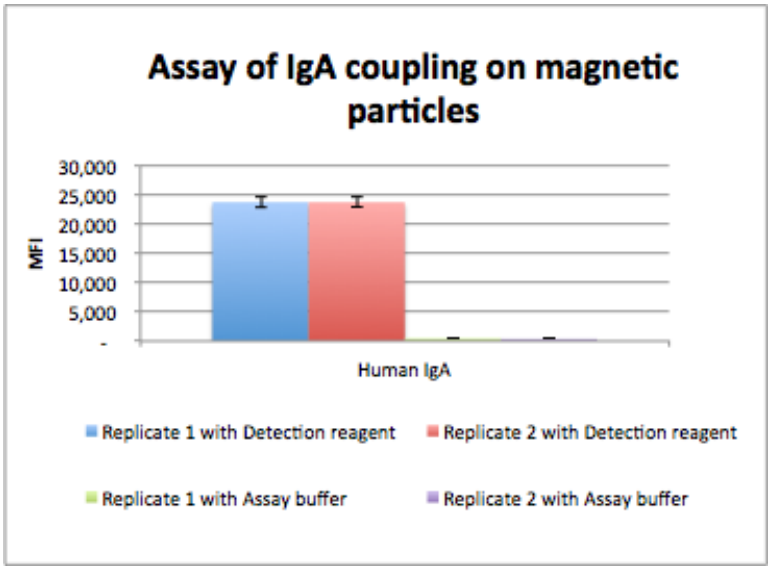


Figure 2: Fluorescent antibody loading assay of IgM coupling on magnetic particles. Replicates showing both specific binding output and blank assay background results for the range of specific RPE labeled detection reagents.

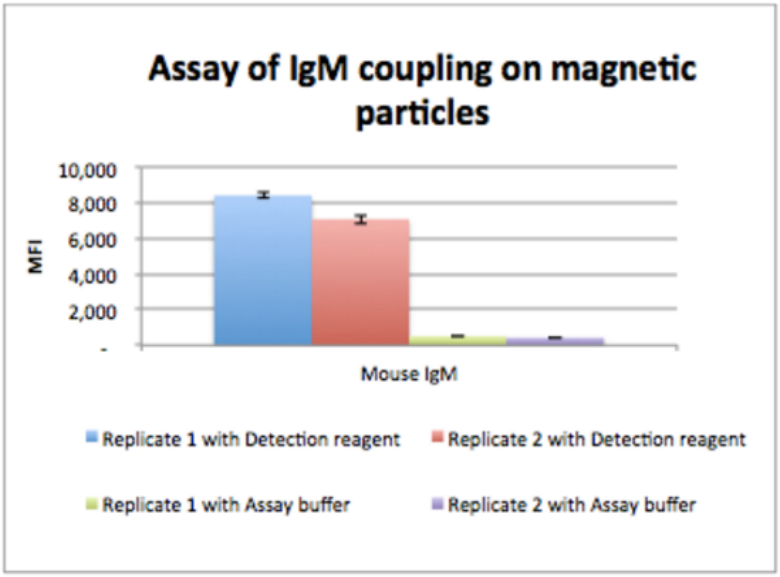
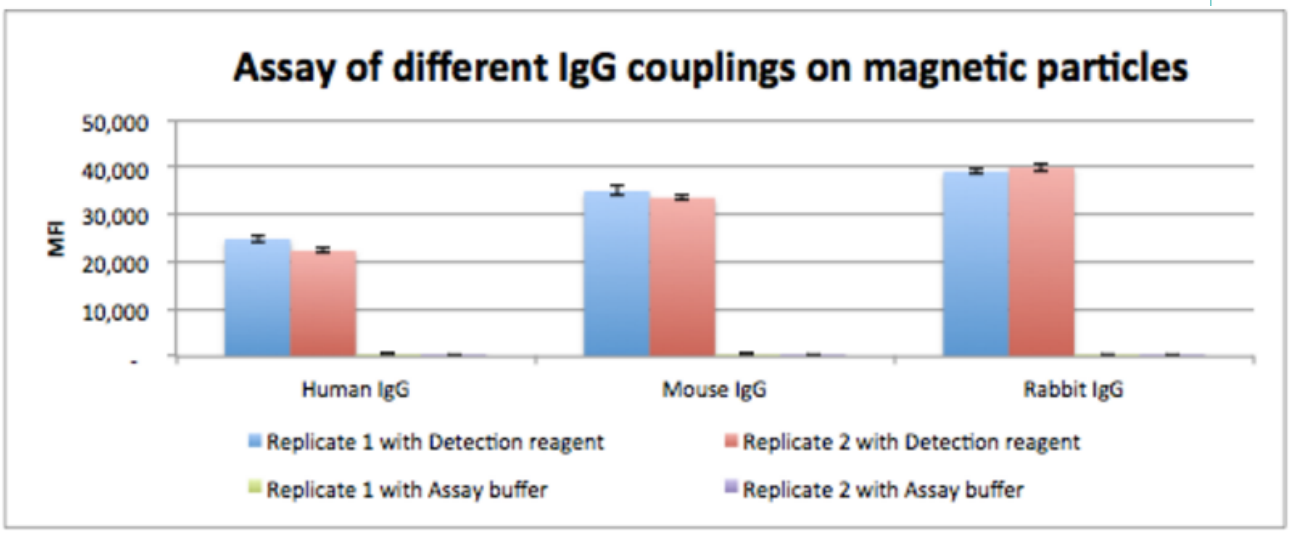


Figure 3: Fluorescent antibody loading assay of IgG coupling on magnetic particles. Replicates showing both specific binding output and blank assay background results for the range of specific RPE labeled detection reagents.



Discussion

Anteo's technology utilises metal polymer complexes. The polymeric nature of the technology allows multi-valent binding of the target antibody through chelation to the electron donating groups of the antibody which results in a gentle yet strong approach to coupling antibodies [2]. In contrast, covalent chemistries offer strong bonds that may impact the antibody structure. Both passive adsorption and covalent chemistries often suffer from loss of activity due to the antibody structure being deformed or the active site of the antibody being shielded [3].

A panel of five different antibodies was selected to cover a wide range of immunoglobulin types (IgA, IgG and IgM) and species sources (mouse, rabbit and human) as shown in figures 1, 2 and 3.. To highlight the flexibility of the Antibody Coupling Kit, all of the antibodies that were tested were coupled at the same antibody concentration and assayed under the same conditions. The antibodies that were used in this experiment had varying primary, secondary and tertiary structure as well as differences in species and antibody class types. The IgG antibodies consists of two heavy polypeptide chains and two light polypeptide chains, this forms a 'Y' shaped monomeric structure. The IgA antibodies are found as a dimer structure, while IgM antibodies exists as a pentamer. At the same volume, the differences in the size of the antibody structure will affect the number of moles of antibody present. As a result, the molar concentrations present in each antibody condition were different, accounting for the differences in signal observed in the loading assays. An example of the differences observed among antibodies is IgG. Between species there is a conserved region through the N-glycosylation site in the heavy chain of the Fc region [4]. The oligosaccharides present at this site are highly heterogenous and have been found to affect the biological, pharmacological and physicochemical properties of IgGs [5]. These subtle differences between antibodies may require further antibody specific optimisation to account for variations in the electron density groups available to co-ordinate to the metal polymer complex.

Conclusion

A wide range of antibodies are used in the life sciences. In the past, the heterogeneous nature of antibodies and their properties often required pre-screening against several binding chemistries to select the most compatible for a given antibody. In contrast, Anteo's Antibody Coupling Kit enables the use of a broad range of different antibodies using a single chemistry.

Traditional covalent binding chemistries use harsh chemicals that may impact the antibody function [6]. Whilst with the use of Anteo's unique technology, antibodies are bound through its multi-component binding property; this gentle approach results in very high antibody function. The binding that is observed with the Antibody Coupling Kit is a highlight of the ease with which a result can be obtained.

The multiple benefits that are associated with using the Antibody Coupling Kit are the ease of use, flexibility to bind any antibody to any particle from 200nm to 3µm magnetic, latex and slica particles, reduction in background, reduced development time, more accurate and reproducible results. Anteo's technology allows scientists to successfully bind any antibody class and eliminates the need for screening other compatible chemistries.

Contact Customer Support: If you have any questions about the Anteo Antibody Coupling Kit, contact, support@anteotech.com or call +61 (0) 7 3219 0085.

Anteo's products are for research use only.

References

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