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A Novel User Friendly Chemistry to Couple Antibodies and Challenging Proteins to Luminex MagPlex Microspheres

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Introduction to Method

The AMG™ Activation Kit for Multiplex Microspheres is based on the novel Mix&Go™ chemistry and was designed to provide researchers and developers with an easy to use method for binding of antibodies and proteins to Luminex® MagPlex® or MicroPlex® beads. Mix&Go is a patented and proprietary metal chelation coordination chemistry that reacts with electron donating groups or oxygenated species on proteins such as carboxylic acids and hydroxyl groups. Since Mix&Go is non-covalent it can bind proteins in a gentle way to preserve biological and conformational activity, yet strongly via multiple chelation binding interactions in an analogous fashion to velcro. The aim of this study was to demonstrate the ease of use of the AMG Activation Kit using IL-8 as a model assay and the ability to successfully bind challenging proteins using formalin inactivated hepatitis A virus (HAV). The activation process is described in Figure 1. A schematic representation of antibody binding to Mix&Go is shown in Figure 2

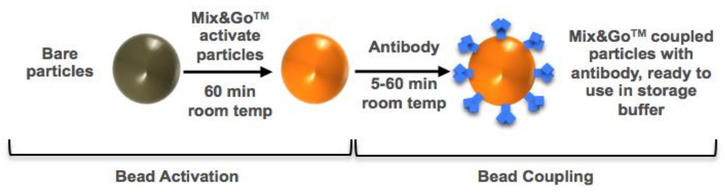


Figure 1. The process of activation and coupling using Mix&Go.

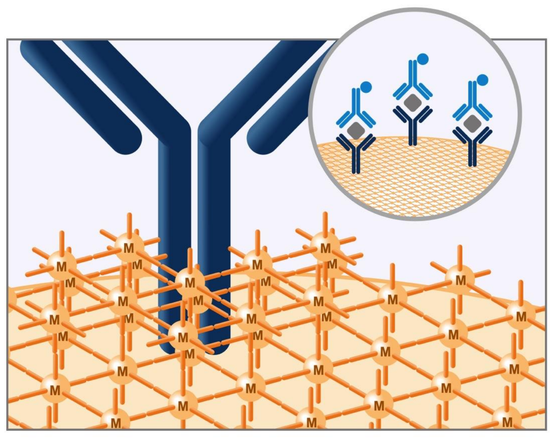


Figure 2. Mix&Go, a molecular glue comprised of polymeric metal ions that chelate to available electron donating groups on synthetic surfaces and proteins.

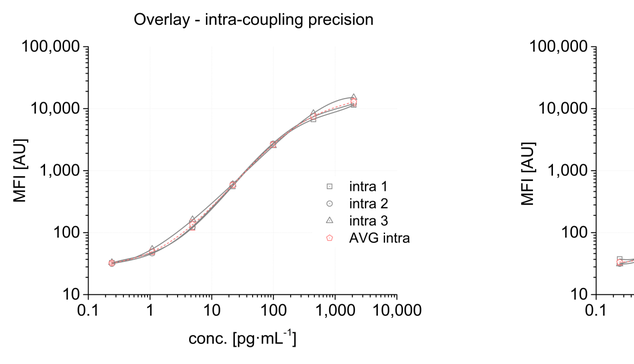
Performance of Coupling Repeatability

To show the performance of the coupling repeatability, beads were activated using the AMG Activation Kit for Multiplex Microspheres in one batch. From this batch several coupling experiments were performed. Anti-IL-8 capture antibody was immobilised on the MagPlex beads, and an IL-8 sandwich immunoassay served as the model system.

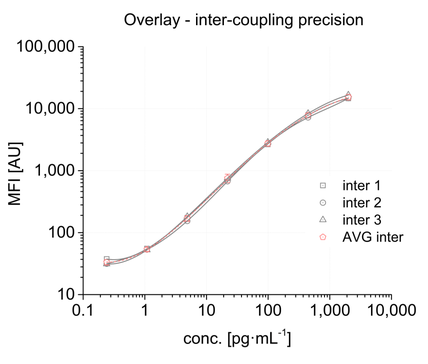
For the intra-day precision three couplings were performed on the same day by one operator. To show the reproducibility between different operators the beads were coupled by three different operators on the same day. The inter-day precision was determined by coupling the antibody on three different days by one operator.

As a read-out, IL-8 immunoassays were performed with standard curves and four human serum samples in two different dilutions. The average and the %CV was calculated using the single results from the duplicate measurements out of the individual runs, and the standard curves are shown in the corresponding graphs (Figure 3). The provided results show an acceptable coupling repeatability.

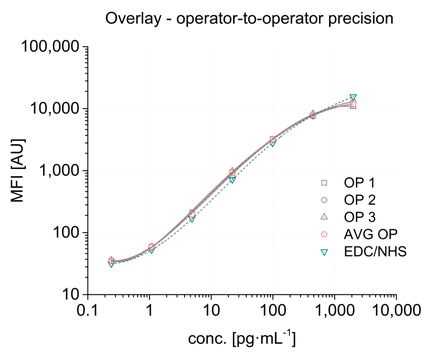
Very similar results were observed with the IL-8 model assay when using the AMG Activation Kit and conventional EDC/NHS coupling protocol.



intra-coupling precision	exp. conc. [pg/ml] / DF	AVG MFI	% CV	AVG conc. [pg/ml]	% CV
S 1	2,000	12,896	13.6	2,012	7.6
S 2	444	7,514	10.9	443	9.1
S 3	98.8	2,637	9.6	103	12.1
S 4	21.9	588	9.5	21.6	8.7
S 5	4.88	136	19.3	4.80	14.8
S 6	1.08	49	9.7	1.11	15.3
S 7	0.24	32	3.7	0.25	12.8
blank	0	27	6.9	-	-
serum 1	1:4	1,307	8.8	48.4	12.1
	1:8	573	12.6	21.2	15.4
serum 2	1:4	630	14.3	23.2	15.8
	1:8	328	12.0	12.2	14.3
serum 3	1:4	396	8.4	14.7	10.4
	1:8	204	4.8	7.47	6.7
serum 4	1:4	403	17.8	14.9	16.9
	1:8	215	12.9	7.91	14.6



inter-coupling precision	exp. conc. [pg/ml] / DF	AVG MFI	% CV	AVG conc. [pg/ml]	% CV
S 1	2,000	15,333	9.8	2,034	20.4
S 2	444	7,806	8.3	448	8.9
S 3	98.8	2,745	9.7	94.5	14.2
S 4	21.9	758	15.5	21.6	13.2
S 5	4.88	170	10.9	4.69	11.8
S 6	1.08	54	7.5	1.08	13.7
S 7	0.24	33	10.2	0.24	35.0
blank	0	27	6.1	-	-
serum 1	1:4	1,435	14.0	43.2	12.9
	1:8	714	23.9	19.9	17.6
serum 2	1:4	1,056	18.0	30.7	18.0
	1:8	477	25.0	13.3	20.0
serum 3	1:4	468	9.9	13.1	8.3
	1:8	261	9.8	7.27	6.8
serum 4	1:4	406	19.2	11.4	20.2
	1:8	218	8.4	6.07	8.3



operator-to-operator precision	exp. conc. [pg/ml] / DF	AVG MFI	% CV	AVG conc. [pg/ml]	% CV
S 1	2,000	11,884	14.2	1,930	59.7
S 2	444	7,914	4.4	445	6.3
S 3	98.8	3,196	9.4	96.2	13.3
S 4	21.9	930	10.1	23.6	17.0
S 5	4.88	197	16.0	4.94	19.2
S 6	1.08	59	12.3	1.04	22.6
S 7	0.24	35	4.0	0.24	23.9
blank	0	28	8.5	-	-
serum 1	1:4	1,173	10.8	30.0	11.0
	1:8	582	15.8	14.7	19.3
serum 2	1:4	779	21.1	19.9	31.0
	1:8	328	15.8	8.17	16.0
serum 3	1:4	403	12.5	10.0	12.8
	1:8	209	12.7	5.17	14.6
serum 4	1:4	393	6.5	9.82	8.7
	1:8	231	27.3	5.71	26.7

Figure 3. Results of coupling repeatability. Standard curves were fitted using a 5-parametric fit. Achieved data are presented in the tables.

Immobilisation of Hepatitis A Virus (HAV)

HAV is the most common cause of acute viral hepatitis. Vaccination against HAV is possible and multivalent vaccines often include HAV.

The diagnosis and vaccination efficacy testing of HAV is by antibody testing. We have immobilised full HAV on beads and measured samples from vaccinated individuals (Figure 5) as well as positive and negative samples (Figure 6).

Experimental set-up

Beads were either activated by the AMG™ Activation Kit for Multiplex Microspheres or following a classical EDC/NHS protocol. Complete, formalin fixed HAV was immobilised at a protein concentration of 20 µg/mL.

For sample measurement, samples were diluted 1:100 in assay buffer and incubated with the beads for 2h at 21°C. After a washing step, bound anti-HAV IgGs were detected by a phycoerythrin labeled gt-anti-hu-IgG antibody (1h at 21°C).

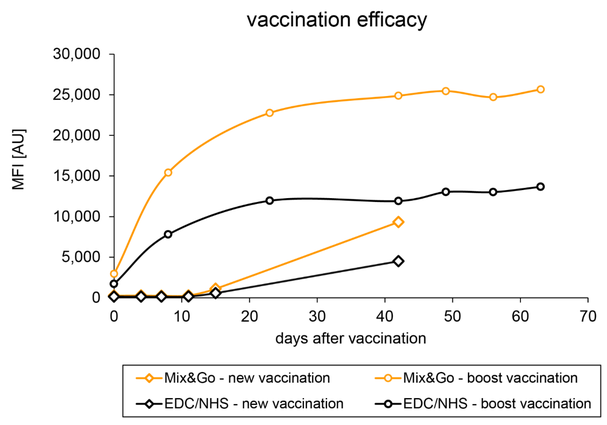


Figure 5. Serological response towards immobilised HAV in samples of a longitudinal study of two vaccinated individuals, a new vaccinated (diamonds) and a booster injection (circle), are shown. The measured MFI values with the Mix&Go bead were double in comparison to EDC/NHS immobilisation of the formalin inactivated HAV.

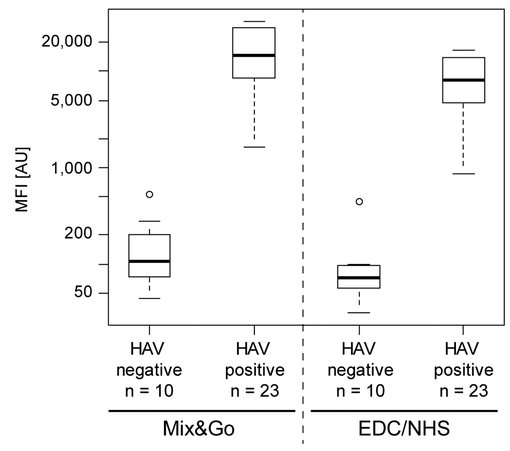


Figure 6. Measurement of patient samples in a serological assay set-up with immobilised HAV.
Results of HAV positive and negative samples demonstrate the possibility for distinct differentiation of HAV positive and negative samples.
MFI values on beads with Mix&Go immobilised HAV are almost double.

Summary & Conclusion

Results demonstrated a very similar IL-8 calibrator response between the AMG Activation Kit and the EDC/NHS. Figure 3 shows that there is low %CV between assays performed on the same days, on different days and also by different operators. This means that the MagPlex microspheres can be easily pre-activated with AMG in a larger batch and used as needed off the shelf, saving time and reducing time to result.

Using the AMG Activation Kit we also showed that inactivated HAV could be functionally immobilised on beads. This can be difficult for EDC/NHS as the inactivation interferes with the amine groups on the protein. Figure 5 shows measured MFI values with the Mix&Go bead in the HAV vaccinated samples were double in comparison to EDC/NHS immobilisation and Figure 6 shows good differentiation between positive and negative HAV samples. This demonstrates how different coupling approaches, such as metal chelation used in Mix&Go, can be used to successfully couple difficult proteins.

We demonstrated the AMG Activation Kit is easy to use, which can contribute to decreased lot-to-lot variation of different assay productions, and provides an alternative chemistry for the immobilisation of challenging proteins.