Covalent coupling of Antibody to MagReSyn® Carboxyl ferromagnetic microparticles using Anteobind™

Method Summary

Part 1: Activation of MagReSyn® Carboxyl microparticles with Anteobind™ Micro

Part 2: Immobilization of Antibody to Activated Beads

Part 3: Modification of Lysine residues on Antibody, protection against proteolytic digestion

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Note: *Part of this protocol is linearly scalable. Should larger or smaller volumes of antibody conjugated beads be required, the protocol can be scaled as per the notes in this protocol.*

Part 1: Activation of MagReSyn® Carboxyl microparticles with Anteobind™ Micro

Materials:

- Anteobind™ Micro solution (Sigma Cat # A-LMPN100-X)
- MagReSyn® Carboxyl ferromagnetic microparticles (20 mg.ml^{-1})

Method:

1. Allow reagents to come to room temperature.
2. Resuspend MagReSyn® Carboxyl magnetic beads and transfer 200 µl (4 mg) to a 2 ml Protein LoBind® tube.
3. Separate the particles from the solution using magnet and remove the supernatant.
4. Add 500 µL of Mix&Go Micro to the tube.
   Note: *Should you require to activate a larger bead amount please keep the ratio of bead to Anteobind™ solution constant.*
5. Mix to ensure the particles are well suspended.
6. Incubate the beads and reagents for 60 minutes at room temperature, keeping particles in suspension using gentle mixing.
7. Separate the particles from the solution using magnet and remove 320 µL supernatant to obtain ~200 µL total volume and a particle suspension of 20 mg/ml
8. The particles are now stably activated with Anteobind™, and can be used for immobilization of antibody immediately, or stored for up to 1 year in the Anteobind™ Micro solution at 4°C.

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Part 2: Immobilization of Antibody to Activated Beads

Materials:

- Anteobind™ activated MagReSyn® Carboxyl ferromagnetic microparticles (Part 1)
- Coating Buffer 25 mM MES buffer pH 6.0:
    - Dissolve 0.489g of MES Hydrate (Sigma-Aldrich M2933) in 90 mL of H_2O, adjust pH to 6 using NaOH. Make up to 100 mL with ddH_2O.

  Note: *please consult relevant literature for alternate buffers that could be used for coupling. Avoid using buffers that contain chelating agents such as phosphate (e.g. PBS) and EDTA*
- Storage Buffer TBS (50 mM Tris-Cl, 150 mM NaCl) pH 8.0
    - Dissolve 1 packet of TBS (Sigma-Aldrich T6664) in 1,000 mL of H_2O. Alternatively, dissolve 0.61 g Tris and 0.88 g NaCl in 80 mL of H_2O. Adjust pH to 8 and make volume up to 100 mL with H_2O

  Note: *PBS or other antibody-compatible buffers could be used for storage of conjugated beads.*
- Antibody, which has been exchanged into a suitable coating buffer.
    - For buffer exchange one could utilize Zeba™ Spin desalting columns (Thermo)
- Quenching/blocking solution: 200 mM Ethanolamine, pH 5.5
    - Dilute 123 µL of ethanolamine (~16.23M, Sigma Aldrich E9508) in ~ 5ml ultrapure water.
    - Adjust the pH to 5.5 by dropwise addition of concentrated HCl.
    - Adjust final volume to a total of 10 ml with ultrapure water

  Note*: Proceed with caution when adjusting pH with conc. HCl. Wear appropriate PPE, and work in a fume cupboard. Allow solution to cool between additions if necessary.*

Method:

1. Vortex the activated particles for 20-30 seconds to form a homogeneous suspension.
2. Transfer 100 µL (2 mg) of particles to a 2 ml protein LoBind® tube.
   Note*: The amounts noted here are guidelines only, based on 10% antibody:particle loading. The ratio of particles to antibody may require optimization.*
3. Separate the particles from the solution using magnet and remove the supernatant.
4. Add 200 to 500 µL of coating buffer to the tube and resuspend the particles.
   Note: *If the particle amount is scaled the required buffer volume needs to be sufficient to cover the microparticles and allow for efficient mixing*
5. Repeat above wash steps (2 - 3).
6. Add the 200 µg of antibody (buffer exchanged into coating buffer) to the particles.
7. Incubate for 60 minutes at room temperature, keeping the particles in suspension.
8. Separate the particles from the solution using magnet and remove the supernatant.
   Note: The supernatant can be checked for unbound antibody by UV spectroscopy (A280nm)
9. Add 200-500 µL of 200 mM Ethanolamine pH 5.5 and mix to form a homogeneous suspension.
10. Incubate for 3 h at RT (or ~12 hours at 4^{o}C), whilst keeping the particles in suspension
11. Apply magnetic separator and aspirate and discard excess quenching agent.

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12. Wash with 200-500 µL of a suitable storage buffer (consult your antibody supplier, e.g. sodium phosphate buffer containing 0.05% sodium azide)
13. Place beads on a magnetic separator, remove supernatant and discard.
14. Add 100 µL storage buffer and store at 4°C. DO NOT FREEZE.

Part 3: Modification of Lysine residues on Antibody, protection against proteolytic digestion

Materials:

- Picolene (2-Methylpyridine borane complex, Sigma Cat# 654213-5G)
- Methanol
- Formalin 10% (Sigma cat# HT50128)
- 10 mM Sodium phosphate pH 7.0

Method:

1. Prepare 0.6 M picolene by dissolving 16 mg picoline in 250 µL methanol – PREPARE FRESH IMMEDIATELY BEFORE USE.
2. Prepare 4% formalin solution by transferring 100 µL 10% formalin to a protein LoBind® tube and adding 150 µL ultrapure water.
3. Equilibrate the particles in 10 mM sodium phosphate buffer by 3 x 200 µL washes using magnetic separation, and removal of the supernatant.
4. Add a suitable volume of 10 mM sodium phosphate to the microparticles to allow for efficient mixing e.g. 200-500 µL.
5. Add 3 µl of 0.6M picolene (from step 1) and 3µL of 4% formalin (from step 2)

   ***Note**: This is the recommended ratio of picolene and formalin per 100 µg antibody. This can be further scaled linearly as per example below.*

Antibody (µg)	Bead Volume (µl) / amount (mg)	10mM Sodium Phosphate (µl)	0.6M Picolene (µl)	4% Formalin (µl)
100	50 µl / 1 mg	200	1.5	1.5
200	100 µl / 2 mg	200	3	3
500	500 µl / 10 mg	500	15	15

   Recommended bead amount is for a 10% antibody loading.

6. Mix for 60 min at room temperature while keeping the particles in suspension. Wash particles with 3 x 100 µL volumes of suitable antibody storage buffer.
7. Suspend particles in a suitable volume of storage buffer to obtain a particle concentration of 10-20 $mg.ml^{-1}$ and proceed to antibody immunoprecipitation experimentation.

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