INSTRUCTIONS FOR USE

Mix&Go™ Micro

Mix&Go Micro is for Laboratory Use Only

Mix&Go Micro and this protocol were developed for binding antibodies and other proteins to magnetic particles greater than 1 µm in size. It forms strong multi-valent interactions with almost any electron donating groups including carboxylic acids commonly found on such particles. These activated particles are storable in various aqueous buffers. However, once any proteins are added, Mix&Go activated particles rapidly form very strong but gentle binding interactions in the order of minutes. Where simplicity, ease of use and high sensitivity are required, Mix&Go Micro has many advantages over existing methods such as EDC/NHS chemistry or alternative "pre-activated" methods such as Epoxy or Tosyl.

For particles 1 µm and smaller the recommended Mix&Go product is Mix&Go Sub-Micron (Cat #: A-SMPN100-X).

Mix&Go Micro can be stored at room temperature and is provided ready-to-use.

This document outlines a general procedure based on M270 Dynabeads, Merck M1 and Bangs ProMag particles using various monoclonal and polyclonal antibodies. However, considering that other surface functional groups and other proteins can be used, some optimisation maybe required when testing different particles and proteins.

Product Description

Anteo Cat #: A-LMPN100-5 (5 mL)
A-LMPN100-10 (10 mL)

pH value: 4.45 – 4.65

Storage: Shelf life at 20°C – 25°C is 3 years
Mix&Go can tolerate temperature conditions from 4°C – 45°C and is not recommended to be exposed to temperatures exceeding 60°C

Compatibility

Helpful Hint:
Avoid using buffers that contain chelating agents such as phosphates (e.g. PBS) and EDTA.

Automation: Many automated apparatuses may contain surfaces that Mix&Go reagent can bind to; therefore the solution should be tested using manual procedures before introduction into an automated system. Take caution of accidental exposure of Mix&Go reagent to bottles, glassware and other surfaces.

Particle Type: Mix&Go Micro has been used to stably activate many particles. Examples include M-270 Dynabeads, Bangs ProMag, JSR MS300 Carboxyl and Estapor M1-200/20.

Buffers:

Coating Buffer 25 mM MES buffer pH 6.0.
Dissolve 4.881g of MES Hydrate (Sigma-Aldrich M2933) in 900 mL of H_2O, adjust pH to 6 using NaOH. Make up to 1,000 mL with H_2O.

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. If packet not available, dissolve 6.05 g Tris and 8.76 g NaCl in 800 mL of H_2O. Adjust pH to 8 and make volume up to 1,000 mL with H_2O

Coating Concentration: The standard concentration range for coating antibody is 250 – 1,000 µg/mL. Antibody coating concentration is best optimised as this can vary depending on the antibody used.

Micro particles vary widely and the following procedure states only general conditions. Additional information may be found on the Anteo Technologies website or by contacting Anteo directly.

Safety Precautions

Standard precautions exercised when handling laboratory reagents should be adhered to. Refer to Material Safety Data Sheet for Mix&Go Micro.

Example Procedure

Materials

- Mix&Go Micro (Cat # A-LMPN100-X)
- Particles larger than 1 µm
- 1.5 mL low binding microcentrifuge tubes
- Pipettes
- Magnetic separator for use with magnetic particles or a centrifuge for use with non-magnetic particles
- Sonication bath
- Antibody
- BSA
- Coating Buffer (25 mM MES pH 6.0)
- Storage Buffer (TBS pH 8.0)
- Tube rotator
- Vortex mixer

Activation of Particles with Mix&Go Micro Reagent

1. Allow reagents to come to room temperature.
2. Resuspend the particles and transfer 5 mg to a 1.5 mL tube.
3. Separate the particles from the solution (using magnet or centrifuge) and carefully remove the supernatant.
4. Add 500 µL of Mix&Go Micro to the tube.
5. Ensure the particles are well suspended and sonicate for 5 minutes.
6. Incubate in Mix&Go reagent for 60 minutes at room temperature, keeping the particles in suspension.
7. The particles are now stably activated with Mix&Go Micro and can be coated with antibody immediately or stored in the Mix&Go Micro solution at 4°C.

Recommendations for Antibody Coating

Helpful Hint:
At this stage do not treat the surface with chemicals or buffers with strong chelation potential as Mix&Go will bind to these agents.

8. Vortex the activated particles for 10 seconds and sonicate for 1 minute.
9. Separate the particles from the solution (using magnet or centrifuge) and carefully remove the supernatant.
10. Add 500 µL of coating buffer to the tube and resuspend the particles.
11. Repeat above wash steps (8 - 10) once.
12. Prepare 500 µL of antibody in coating buffer in a fresh 1.5 mL tube.
13. Separate the particles from the solution (using magnet or centrifuge) and carefully remove the supernatant from the tube.
14. Add the 500 µL of antibody from step 12 to the tube.

15. Incubate for 60 minutes at room temperature, keeping the particles in suspension.

Optional Blocking

16. Prepare 500 µL of blocking solution by dissolving 1% w/v BSA in coating buffer
17. Separate the particles from the solution (using magnet or centrifuge) and carefully remove the supernatant.
18. Add 500 µL of the blocking solution, from step 16, to the tube and resuspend the particles.
19. Incubate for 60 minutes at room temperature, keeping the particles in suspension.

Storage of Coated Particles

20. Separate the particles from the solution (using magnet or centrifuge) and carefully remove the supernatant.
21. Add 500 µL of storage buffer to the tube and resuspend the particles.
22. Repeat above wash steps once.
23. Vortex the coupled particles for 10 seconds and sonicate for 1 minute.
24. Store in storage buffer at 4°C

Observations

Particles are stored in Mix&Go Micro until required for antibody coupling. The particles should not form aggregates. This can be observed under a light microscope. Depending on the size and density of the particle, they may settle out with time. This is normal and the particles may be resuspended using a vortex mixer.

Mix&Go reagent allows antibodies and other proteins to bind faster and with more functionality. In some cases, surfaces may be activated with Mix&Go in as little as 5 minutes and proteins can bind to Mix&Go activated surfaces in as little as 10 minutes at room temperature. Some optimisation of incubation times and protein concentration is recommended to utilise the full advantage that Mix&Go has to offer.

Mix&Go Micro should not appear to change colour or form precipitates. If any colour changes or precipitate is observed consult the website for trouble shooting information.

Citations

When describing this product or a procedure using this product, please refer to it as Mix&Go™ Micro from Anteo Technologies.

Technical Support

For questions regarding this product or for technical support please refer to our website or contact us via one of the following methods:

Anteo Technologies Pty Ltd
Unit 4, 26 Brandl Street
Eight Mile Plains
QLD 4113
Australia

Office: (07) 3219 0085
Fax: (07) 3219 0553
Email: support@anteotech.com
www.anteotech.com

Other Mix&Go™ Scientific Products

For further information on other Mix&Go products please refer to our website:

www.anteotech.com