



## Mix&Go™ Sub-Micron Instructions For Use

Mix&Go™ Sub-Micron stably activates carboxylated particles ranging from 0.5 to 1 µm in size. The activated particles are storable and can be used to conjugate antibodies, other proteins either immediately or months later. This reagent is of benefit when assessing different particles, when reproducibility is important between batches or in scaling up.

### Product Description

Catalogue #: A-SMPN100  
 Shelf Life: 36 months from date of manufacture  
 Storage: 20° to 25°C

**Note:** The product is not guaranteed DNase, RNase or endotoxin free.

### Provided Materials

**A-SMPN100-5 or A-SMPN100-10 Mix&Go™ Sub-Micron**  
 Protein conjugation of carboxylated particles.

### Materials Required

- Conjugation Buffer – 25mM MES pH 6.0**  
 Washing Buffer, and as protein diluent.
- Blocking Buffer – 1% BSA diluted in Conjugation Buffer**  
 Blocking solution for conjugated particles.
- Storage Buffer – 50mM TBS pH 8.0, 0.05% ProClin 300**  
 Stable storage of protein conjugated particles.

### Specifications

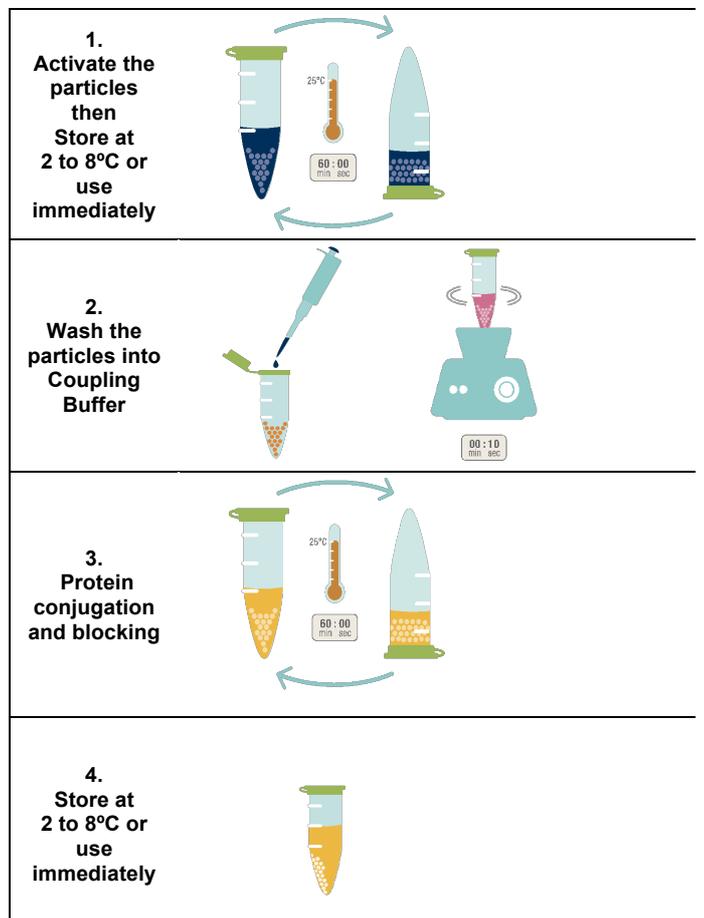
- Applications** Compatible with carboxylated particles from 0.5 to 1 µm in size.
- Safety** Standard safety precautions exercised when handling laboratory reagents should be adhered to. Refer to the product MSDS for safety precautions.
- Regulatory** For laboratory use only

### Important Product Information

- Buffer compatibility** For conjugation step, other slightly acidic buffers (pH 5.0 - 6.5) may be used: Acetate, Hepes or Tris Buffer. Avoid using buffers or additives with strong chelation potential (e.g. PBS, EDTA). For storage of protein conjugates, use recommended Storage Buffer. Avoid addition of protein blockers in the Storage Buffer. For performing assays, PBS buffer, EDTA or additives with strong chelation potential are compatible.
- Reaction tubes** Use of low-binding polypropylene microcentrifuge tubes is recommended.
- Temperature** Do not freeze or expose to temperatures exceeding 60°C. Room temperature is defined as 20°C - 25°C.

<b>Protein concentration</b>	The recommended concentration range for conjugation of antibody is 20 to 50 µg of protein per mg of particles. Optimal protein concentration may vary depending on particle, protein and assay. Dilute antibody stock solution to target concentration with Conjugation Buffer.
<b>Particle Separation</b>	For magnetic particles, magnet strength and particle size will affect separation times. For non-magnetic particles, centrifugation is required. Centrifugation time and speed will vary depending on particle size. Separation is complete when supernatant becomes clear.
<b>Particle aggregation</b>	If particle aggregation is present, vortex-mix and sonicate the particles until adequately dispersed. Anteo recommends using a sonication bath filled with fresh deionised and degassed water.
<b>Scale</b>	This protocol is scalable from 100 µL to 10 mL reaction volumes (1 to 100 mg of particles). Reaction volumes should be selected as appropriate to individual requirements. Note that vessels used, method particulars such as mixing and disaggregation techniques may also require consideration and optimisation. Please consult Anteo Technical Support for recommendations.
<b>Particles</b>	Mix&Go Sub-Micron has been used to stably activate many particles. Examples include MyOne™ Dynabeads™, Merck Estapor® M1 and EM1 particles.

### Procedure Summary



## Procedure

This procedure outlines a general protocol to prepare 5 mg of protein-conjugated magnetic particles. For non-magnetic particles this procedure can be modified with centrifugation taking the place of magnetic separation.

### **i** Helpful Hint:

Ensure reagents are at room temperature before use. Always use a pipette to remove supernatants, taking care not to disturb the particle pellet.

Particles should not be left on magnet for extensive periods of time. When supernatant is clear, solution should be removed and Particles removed from magnet.

## Activation of Magnetic Particles

1. Before taking an aliquot, vortex-mix the bottle of stock particles for 30 seconds to ensure stock particles are resuspended.
2. Transfer 5 mg of the particles (50  $\mu$ L if Stock is 100 mg/mL) to a tube containing 450  $\mu$ L of Mix&Go™ Sub-Micron.
3. Vortex-mix the particles for 10 seconds followed by sonication for 5 minutes.  
Note! If the Stock is less concentrated, transfer 5 mg of particles to a tube. Separate the particles on a magnetic separator, and remove most of supernatant to give a 100 mg/mL (approx. 50  $\mu$ L) particles solution.
4. Incubate the particles on a tube rotator or roller at 50 rpm for 60 minutes at room temperature (20 to 25°C).

Once activated, particles can be conjugated immediately with protein. Alternatively, particles can remain stored in Mix&Go™ Sub-Micron for later use. Store at 2 to 8°C in a low-binding polypropylene tube.

### **i** Helpful Hint:

If multiple protein conjugations are being performed we recommend a single activation of a larger volume of particles. Activated particles are stable for months when stored in this ready-to-use state.

After prolonged storage, suspend particles thoroughly before use (e.g. vortex-mix the particles for 10 seconds, followed by sonication for 5 minutes).

## Protein Conjugation

5. Prepare protein to be conjugated to the particles at the required final concentration in 250  $\mu$ L of Conjugation Buffer in a fresh tube and mix thoroughly.

### **i** Helpful Hint:

For example: to couple at 25  $\mu$ g of protein per mg particles, prepare 250  $\mu$ L of 500  $\mu$ g/mL protein.

6. Separate the activated particles on a magnetic separator, and remove all the supernatant when it becomes clear.
7. Resuspend the particles in 500  $\mu$ L of Conjugation Buffer by vortex-mixing the particles for 10 seconds.
8. Repeat above wash steps (6-7) once.
9. Separate the activated particles on a magnetic separator, and remove all the supernatant when it becomes clear.
10. Resuspend the particles in 250  $\mu$ L of Conjugation Buffer.
11. Vortex-mix the particles for 10 seconds.

12. Add 250  $\mu$ L of the particles into 250  $\mu$ L of prepared antibody solution.
13. Incubate the particles on a tube rotator or roller at 50 rpm for 60 minutes at room temperature (20 to 25°C).

## Blocking the Protein Conjugated Particles

14. Add 50  $\mu$ L of Blocking Buffer into conjugated particles.
15. Vortex-mix the particles for 10 seconds followed by sonication for 5 minutes.
16. Incubate the particles on a tube rotator or roller at 50 rpm for 60 minutes at room temperature (20 to 25°C).

### **i** Helpful Hint:

Anteo suggests BSA as the blocker. You may consider using a lower or higher BSA concentration according to your assay system. The best blocker may vary between different particles, proteins and assay systems. If you experience high non-specific binding, please consider other options such as casein, fish skin gelatin and synthetic blockers.

## Storage of Protein Conjugated Particles

17. Separate the particles on a magnetic separator and remove all the supernatant when it becomes clear.
18. Resuspend the particles in 500  $\mu$ L of Storage Buffer by vortex-mixing the particles for 10 seconds.
19. Repeat above wash steps (17-18) once.
20. Separate the particles on a magnetic separator and remove all the supernatant when it becomes clear.
21. Finally resuspend the particles in 500  $\mu$ L of Storage Buffer.
22. Vortex-mix the particles for 10 seconds, followed by sonication for 5 minutes and vortex-mix for a further 10 seconds. Repeat this step as required to disperse particles if aggregation is observed.

The protein-conjugated particles are now ready for use. Store at 2 to 8°C in a low-binding polypropylene tube if not required for immediate use. Always resuspend particles thoroughly before use (follow step 22).

## For more information

**Frequently Asked Questions** Refer to [www.anteotech.com](http://www.anteotech.com)

**Technical Support** [support@anteotech.com](mailto:support@anteotech.com)

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