anteo technologies

Mix&Go™ Micro
Instructions For Use

Mix&Go™ Micro stably activates carboxylated particles greater than 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-LMPN100

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-LMPN100-5 or A-LMPN100-10 Mix&Go™ Micro
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.

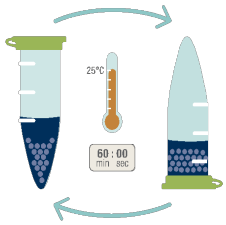
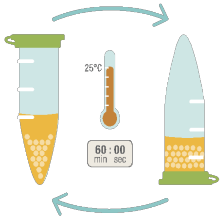
Specifications

Applications	Compatible with carboxylated particles greater than 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 60ºC. Room temperature is defined as 20ºC - 25ºC.
Protein concentration	The recommended concentration range for conjugation of antibody is 10 to 30 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.
Particle Separation	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 and density. Separation is complete when supernatant becomes clear.
Particle aggregation	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.
Scale	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.
Particles	Mix&Go™ Micro can be used to conjugate proteins to many particles. Examples include M-270 Dynabeads™, Bangs ProMag®, JSR MS300 Carboxyl and Merck Estapor® M1-200/20.

Procedure Summary

1. Activate the particles then Store at 2 to 8ºC or use immediately	
2. Wash the particles into Conjugation Buffer	
3. Protein conjugation and blocking	
4. Store at 2 to 8ºC or use immediately	

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.

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 µL if Stock is 100 mg/mL) to a tube containing 450 µL of Mix&Go™ Micro.
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 µL) particles solution.
4. Incubate the particles on a tube rotator or roller at 50 rpm for 60 minutes at room temperature (20 to 25ºC).

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

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 µL of Conjugation Buffer in a fresh tube and mix thoroughly.

Helpful Hint:
For example: to couple at 25 µg of protein per mg particles, prepare 250 µL of 500 µg/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 µL 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 µL of Conjugation Buffer
11. Vortex-mix the particles for 10 seconds.
12. Add 250 µL of the particles into 250 µL 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 25ºC).

Blocking the Protein Conjugated Particles

14. Add 50 µL 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 25ºC).

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 µL 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 µL 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 8ºC in a low-binding polypropylene tube if not required for immediate use. Always resuspend particles thoroughly before use (follow step 22).

For more information

Refer to www.anteotech.com

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