anteo technologies

AMG Coupling Kit, 1 µm Magnetic Particles
Instructions For Use

This AMG™ Coupling Kit contains particles that are fully activated with Anteo's Technology and are ready to use, with buffer solutions known to work for a majority of applications. Protocols should be optimised to meet individual requirements.

Product Description

Catalogue #: A-SMPCKMP

Shelf Life: 12 months from date of manufacture

Storage: 2 to 8°C (do not freeze)

Note: This product contains ProClin 300 as a preservative. The product is not guaranteed DNase, RNase or endotoxin free.

Provided Materials

A-CMPAPMP Activated 1 µm Particle
Ready-to-use particles prepared with Anteo's Technology.

A-CMPCBA1 Coupling Buffer
Equilibration of activated particles for coupling, and as protein diluent, pH 6.0.

A-CMPBBA1 Blocking Buffer
Concentrated blocking solution for coupled particles, pH 6.0.

A-CMPSBA1 Storage Buffer
Stable storage of protein coupled particles, pH 8.0.

1.5 mL Microcentrifuge Tubes
Consumable to be use during coupling procedure.

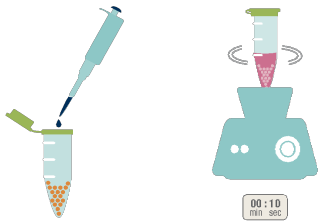
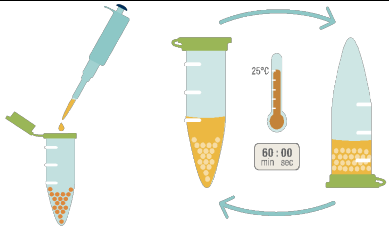
Specifications

Supplied Surface	Particle: 1 µm, Superparamagnetic, 40% Magnetite. Concentration: 10 mg/mL (1% w/v solids).
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	This product is not compatible with concentrations of phosphates >2.5 mM. During coupling it is recommended to dilute the phosphate concentration with the provided Coupling Buffer to <2.5 mM.
Reaction tubes	Use of low-binding polypropylene microcentrifuge tubes is recommended (provided).
Temperature	Allow reagents to equilibrate to room temperature before use. Store this product at 2 to 8ºC when not in use. Do not freeze particles or expose to temperatures exceeding 60ºC.
Protein concentration	The user should optimise protein-coupling concentration as this can vary depending on protein type and particle type. The recommended concentration range for coupling antibody is 25 to 100 µg of protein per mg of particles, with a determined maximal binding capacity up to 30 µg/mg particles.
Particle Separation	For magnetic particles, magnet strength and particle size will affect separation times. Separation is complete when supernatant becomes clear. This step can take up to 5 minutes.
Particle aggregation	It is recommended to confirm that the particles are dispersed by microscope or Dynamic Light Scattering (DLS) measurements. The degree of aggregation may be particle dependent. If aggregated, 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 maximum volume of particles provided in the kit. 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.
Binding Capacity (Mouse IgG)	> 20µg/mg particles
Monodispersity	> 90%

Procedure Summary

1. Wash the activated particles into Coupling Buffer	
2. Protein coupling and blocking	
3. Store at 2 to 8ºC or use immediately	

Procedure

This procedure outlines a general protocol to couple protein to 1 mg of magnetic particles. Note that vessels used, method particulars such as mixing and disaggregation techniques may also require consideration and optimisation.

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.

Preparation of Diluted Protein

1. Prepare protein to be coupled to the particles at the required final concentration in 50 µL of Coupling Buffer in a fresh tube and mix thoroughly by vortex-mixing. Note that the coupling step is performed at 10 mg/mL particles final concentration.

Helpful Hint:
For example: to couple at 50 µg of protein per mg particles, prepare 50 µL of 1000 µg/mL protein.

Protein Coupling

2. Before taking an aliquot, vortex-mix the bottle of stock particles for 10 seconds, followed by sonication for 1 minute to ensure stock particles are resuspended.
3. Transfer 100 µL (1 mg) of magnetic particles to a provided 1.5 mL tube.
4. Separate the activated particles on a magnetic separator, and remove all the supernatant when it becomes clear.
5. Resuspend the particles in 100 µL of Coupling Buffer by vortex-mixing the particles for 10 seconds.
6. Repeat above wash steps (4-5) twice.
7. Resuspend the particles in 50 µL of Coupling Buffer.
8. Add all the particles to the prepared diluted protein solution.
9. Vortex-mix the particles for 10 seconds, followed by sonication for 5 minutes and vortex-mix for 10 seconds.
10. Incubate for 60 minutes at room temperature (20 to 25ºC) on a tube rotator or roller.

Blocking the Protein Coupled Particles

11. Add 10 µL of the Blocking Buffer to the tube containing the particles and mix thoroughly by vortex-mixing.
12. Incubate for 60 minutes at room temperature (20 to 25ºC) on a tube rotator or roller.

Storage of Protein Coupled Particles

1. Separate the particles on a magnetic separator and remove all the supernatant when it becomes clear.
2. Resuspend the particles in 100 µL of Storage Buffer by vortex-mixing the particles for 10 seconds.
3. Repeat above wash steps (22-23) once.
4. Separate the particles on a magnetic separator and remove all the supernatant when it becomes clear.
5. Finally resuspend the particles in 100 µL of Storage Buffer.
6. 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.

Helpful Hint:
Check particles at this stage for aggregation.
Take care during repeated and prolonged sonication as the sonication or temperatures produced during this may damage the protein. Replace the water, or iced-water in the sonication bath often to reduce the temperature and monitor as required.

The protein-coupled particles are now ready for use at a final concentration of 10 mg/mL. Store at 2 to 8ºC if not required for immediate use. Resuspend particles before use (step 18).

For more information

Reordering	Refer to www.anteotech.com
Technical Support	support@anteotech.com