anteo technologies

Lateral Flow Coupling Kit For Magnetic And Latex Particles
Instructions For Use

This Lateral Flow Coupling Kit utilises Anteo's patented technology to activate and couple proteins of interest to carboxylated magnetic and latex particles ranging from 200 nm to 500 nm in size.

Product Description

Catalogue #: A-VMPLFMP

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-CMPPPS1 Particle Pretreatment Solution
Pretreatment of particles before activation, contains surfactant.

A-CMPPAS1 Particle Activation Solution
Activation of carboxylated particles with Anteo's Technology.

A-CMPPIS1 Particle Intermediate Solution
Storage of activated particles prior to coupling.

A-CMPCBC1 Coupling Buffer
Equilibration of activated particles for coupling, and as protein diluent, pH 6.0.

A-CMPBBB2 Blocking Buffer
Concentrated blocking solution for coupled particles, pH 6.0.

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

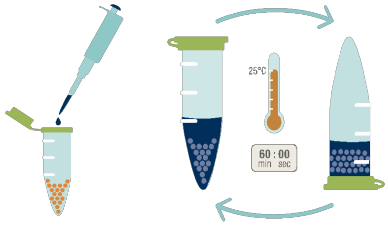
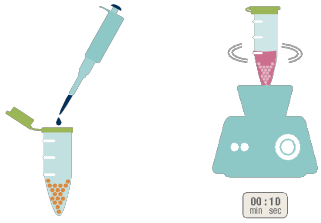
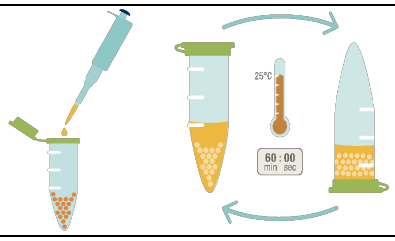
Specifications

Applications	Compatible with carboxylated particles from 200 nm to 500 nm 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	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.
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.
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. Separation is complete when supernatant becomes clear.
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 5 mL reaction volumes (1 to 50 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.

Procedure Summary

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

Procedure

This procedure outlines a general protocol to prepare 1 mg of protein-coupled magnetic particles. For non-magnetic particles this procedure can be modified accordingly.

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.

Pre-treatment of Magnetic Particles

1. Before taking an aliquot, vortex-mix the bottle of stock particles for 30 seconds, followed by sonication for 5 minutes and a further vortex-mix for 30 seconds to ensure stock particles are resuspended.
2. Transfer 1 mg magnetic particles to a tube.
3. Separate the particles on a magnetic separator, and remove all the supernatant when it becomes clear.
4. Resuspend the particles in 10 µL of Particle Pretreatment Solution. Vortex-mix for 10 seconds.

Activation of Magnetic Particles

5. Add 90 µL of Particle Activation Solution to a new tube.
6. Quickly dispense the particles at a constant rate into the Particle Activation Solution.
7. Vortex-mix the particles for 10 seconds followed by sonication for 5 minutes and a further vortex-mix for 10 seconds.
8. Incubate the particles on a tube rotator or roller for 1 hour at room temperature (20 to 25ºC).
9. Separate the particles on a magnetic separator, and remove all the supernatant when it becomes clear.
10. Resuspend the particles in 100 µL of Particle Intermediate Solution.
11. Vortex-mix the particles for 10 seconds, followed by sonication for 5 minutes and a further vortex-mix for 10 seconds.

Activated particles are now at 10 mg/mL and ready for immediate protein coupling, or can be stored at 2 to 8ºC.

Helpful Hint:
If particles have been stored in Particle Intermediate Solution, it is recommended to resuspend the particles by vortex-mixing for 10 seconds and sonication for 5 minutes and vortex-mixing for 10 seconds. Check particles at this stage for aggregation.

Preparation of Diluted Protein

12. Prepare protein to be coupled to the particles at the required final concentration in 100 µL of Coupling Buffer in a fresh tube and mix thoroughly by vortex-mixing. Note that the coupling step is performed at 5 mg/mL particles final concentration.

Helpful Hint:
For example: to couple at 50 µg of protein per mg particles, prepare 100 µL of 500 µg/mL protein.

Protein Coupling

13. Separate the activated particles on a magnetic separator, and remove all the supernatant when it becomes clear.
14. Resuspend the particles in 100 µL of Coupling Buffer by vortex-mixing the particles for 10 seconds.
15. Repeat above wash steps (13-14) once.
16. Resuspend the particles in 100 µL of Coupling Buffer.
17. Add all the particles to the prepared diluted protein solution.
18. Vortex-mix the particles for 10 seconds, followed by sonication for 5 minutes and vortex-mix for 10 seconds.
19. Incubate for 60 minutes at room temperature (20 to 25ºC) on a tube rotator or roller.

Blocking the Protein Coupled Particles

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

Storage of Protein Coupled Particles

22. Separate the particles on a magnetic separator and remove all the supernatant when it becomes clear.
23. Resuspend the particles in 200 µL of Storage Buffer by vortex-mixing the particles for 10 seconds.
24. Repeat above wash steps (22-23) once.
25. Separate the particles on a magnetic separator and remove all the supernatant when it becomes clear.
26. Finally resuspend the particles in 100 µL of Storage Buffer.
27. 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 27).

For more information

Frequently Asked Questions	Refer to www.anteotech.com/FAQ
Technical Support	support@anteotech.com