anteo technologies

Universal Coupling Kit
Instructions For Use

This Universal Coupling Kit can activate and couple proteins of interest to carboxylated particles ranging from 200 nm to 3 µm in size. The activated particles can be used to couple antibodies and other proteins either immediately or can be stored for many months without change to their protein coupling efficiency. This kit is of benefit when assessing different particles and conjugating multiple proteins during optimisation and for the development of various immunoassay or bio-separation applications.

Product Description

Catalogue #: A-VMPAKMP

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-CMPBBB3 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 3 um 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

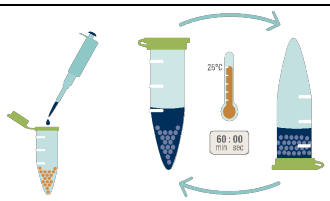
Protein compatibility	This product forms a highly activated surface for coupling proteins such as antibodies, streptavidin, Protein A/G, and fluorescent proteins etc. It is not designed for the coupling of short peptides or small molecules.
Reaction tubes	Use of low-binding polypropylene microcentrifuge 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 and size. The recommended concentration range for coupling antibody is 10 to 125 µg of protein per mg of particles. The recommended starting concentration for particle >0.5 µm is **20 µg** of protein per mg of particles. The recommended starting concentration for particle <0.5 µm is **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 and density. For further details please refer to the particle manufacturer's recommendations. Separation is complete when supernatant becomes clear.
Particle aggregation	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 contact Anteo Technical Support for recommendations.

Procedure Summary

1.
Activate the pretreated particles with **Particle Activation Solution**

Wash with **Particle Intermediate Solution**

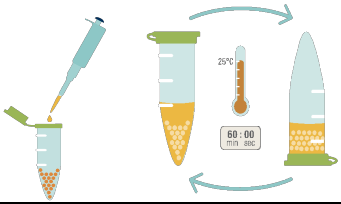
Store at 2 to 8ºC or use immediately



2.
Wash the particles with **Coupling Buffer**

3.
Protein coupling in **Coupling Buffer**

and blocking with **Blocking Buffer**



4.
Wash with **Storage Buffer**

Store at 2 to 8ºC or use immediately

Example Procedure For Magnetic Particles

This procedure outlines a general protocol to prepare **1 mg** of protein-coupled magnetic particles. For non-magnetic particles centrifugation steps can be used in place of magnetic separation. Please refer to the particle manufacturer's recommendation for centrifugal specifications.

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 all particles (from step 4) 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 gently (e.g. 50 rpm) 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:
Activated particles tested at Anteo are stable for 12 months in Particle Intermediate Solution. After prolonged storage, suspend particles thoroughly before use (e.g. vortex-mix the particles for 10 seconds, followed by bath sonication for 5 minutes). *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. For example: to couple at 50 µg of protein per mg particles, prepare 100 µL of protein at a concentration of 500 µg/mL.

Protein Coupling

13. Separate the 100 µL activated particles (from step 11) 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. Add 100 µL of particles to the prepared 100 µL of diluted protein solution (from step 12). Note that the coupling step is performed at 5 mg/mL particles final concentration.
17. Vortex-mix the particles for 10 seconds, followed by sonication for 5 minutes and vortex-mix for 10 seconds.
18. Incubate for 60 minutes at room temperature (20 to 25ºC) on a tube rotator or roller gently (e.g. 50 rpm).

Blocking the Protein Coupled Particles

19. Add 20 µL of the Blocking Buffer to the tube containing the 200 µL of particles from step 18, and mix thoroughly by vortex-mixing.
20. Incubate for 60 minutes at room temperature (20 to 25ºC) on a tube rotator or roller gently (e.g. 50 rpm).

Helpful Hint:
Anteo's Blocking Buffer contains high concentration of BSA. For particle size greater than 1 µm, if lower than expected signals is observed please consider using ≤ 4 µL of Blocking Buffer instead of 20 µL in the step 19. 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 or synthetic blockers. Please prepare your specific Blocking Buffer in Coupling Buffer provided before use.

Storage of Protein Coupled Particles

21. Separate the particles on a magnetic separator and remove all the supernatant when it becomes clear.
22. Resuspend the particles in 200 µL of Storage Buffer by vortex-mixing the particles for 10 seconds.
23. Repeat above wash steps (21-22) once.
24. Separate the particles on a magnetic separator and remove all the supernatant when it becomes clear.
25. Finally resuspend the particles in 100 µL of Storage Buffer.
26. 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-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 26).

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.

For more information

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