anteo technologies

AMG™ Activation Kit for Multiplex Microspheres
Instructions For Use

This AMG™ Activation Kit utilises Mix&Go™ technology to stably couple proteins of interest to multiplex microspheres e.g. Luminex® MagPlex® or MicroPlex®. Protocols should be optimised to meet individual requirements.

Product Description

Catalogue #: A-LMPAKMM

Shelf Life: 21 months from date of manufacture (refer to label)

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-CMPARA1 Mix&Go™ Activation Reagent
Particle activation solution.

A-CMPCBB1 Coupling Buffer
Equilibration of activated microspheres for coupling, and as protein diluent, pH 5.2.

A-CMPSBB1 Storage Buffer
Stable storage of protein coupled microspheres.

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

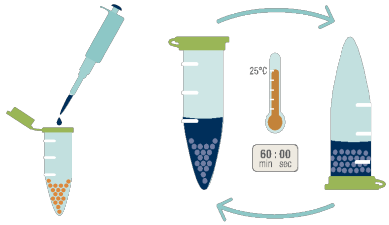
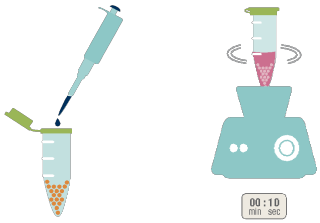
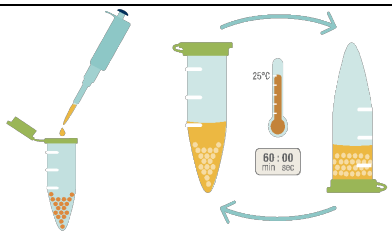
Specifications

Applications	Compatible with multiplex microspheres such as Luminex® MagPlex® and MicroPlex® microspheres within assays developed for the Luminex® xMAP® instrument platform.
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 microspheres 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 starting concentration for coupling antibody is 25 µg of protein per 12.5 million (12.5 x 10^6) of microspheres.
Particle Separation	For magnetic microspheres, magnet strength and particle size will affect separation times. Separation is complete when supernatant becomes clear. This step can take up to 5 minutes. This kit is also compatible with non-magnetic microspheres. To separate non-magnetic microspheres use a centrifuge at manufacturer's recommended speeds (e.g. 8,000 rcf for 5 minutes).
Scale	This protocol is scalable. 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 microspheres	
2. Wash the microspheres 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 couple protein to 1.25 million of magnetic multiplex microspheres. Note that vessels used, method particulars such as particle separation and mixing 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.

Activation of Magnetic Microspheres

1. Resuspend microspheres by gentle inversion for 1 minute.
2. Aliquot 100 µL of the microsphere suspension (concentration: 12.5 million (12.5×10^6) microspheres/mL) to a provided 1.5 mL tube.
3. Separate the activated microspheres on a magnetic separator, and remove all the supernatant when it becomes clear.
4. Resuspend the microspheres in 100 µL of Mix&Go Activation Reagent.
5. Vortex-mix the microspheres for 10 seconds.
6. Incubate the microspheres on a tube rotator or roller for 60 minutes at room temperature (20ºC to 25ºC).

Activated microspheres are now ready for immediate protein coupling, or can be stored at 2 to 8ºC.

Preparation of Diluted Protein

7. Prepare protein to be coupled to the microspheres at the required final concentration in 100 µL of Coupling Buffer in a fresh tube and mix thoroughly by vortex-mixing.

Protein Coupling

8. Vortex-mix the bottle of activated microspheres for 10 seconds, followed by sonication for 1 minute to ensure stock microspheres are resuspended.
9. Separate the activated microspheres on a magnetic separator, and remove all the supernatant when it becomes clear.
10. Resuspend the microspheres in 100 µL of Coupling Buffer by vortex-mixing the microspheres for 10 seconds.
11. Repeat above wash steps (9-10) once.
12. Separate the activated microspheres on a magnetic separator, and remove all the supernatant when it becomes clear.
13. Resuspend the microspheres in 100 µL of prepared antibody solution.
14. Vortex-mix the microspheres for 10 seconds.
15. Incubate the microspheres on a tube rotator or roller for 60 minutes at room temperature (20ºC to 25ºC).

(Optional) Blocking the Protein Coupled Microspheres

Helpful Hint:
0.1% (w/v) Bovine Serum Albumin (BSA) in Coupling Buffer can be used as a blocker.

16. Separate the activated microspheres on a magnetic separator, and remove all the supernatant when it becomes clear.
17. Resuspend the microspheres in 100 µL of Coupling Buffer by vortex-mixing the microspheres for 10 seconds.
18. Repeat above wash steps (16-17) once.
19. Prepare 100 µL of blocker in Coupling Buffer in a fresh tube.
20. Separate the activated microspheres on a magnetic separator, and remove all the supernatant when it becomes clear.
21. Resuspend the microspheres in 100 µL of blocker and mix thoroughly by vortex-mixing.
22. Incubate the microspheres on a tube rotator or roller for 60 minutes at room temperature (20ºC to 25ºC).

Storage of Protein Coupled Microspheres

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

Helpful Hint:
Check microspheres 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 microspheres are now ready for use. Store at 2 to 8ºC if not required for immediate use. Resuspend microspheres by vortex-mixing before use.

For more information

Re-ordering	Refer to www.anteotech.com
Technical Support	support@anteotech.com