AMG Coupling Kit, 1 µm Magnetic Particles

AI-SMPCKMP-04.00

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

Materials Supplied	
Cat. No.	Component
A-CMPAPMP	Activated 1 µm Magnetic Particles
A-CMPCBA1	Coupling Buffer
A-CMPBBA1	Blocking Buffer
A-CMPSBA1	Storage Buffer
n/a	1.5 mL microcentrifuge tubes

Additional Materials Required
Protein of interest
Pipettes
Magnetic separator for tubes
Tube rotator
Vortex mixer
Microcentrifuge for 1.5 mL tubes
Sonication bath with fresh deionised water (minimum power 60W)

Specifications	
Ordering Information	A-SMPCKMP-10 (10 Reactions) A-SMPCKMP-30 (30 Reactions)
Storage	Store at 2°C - 8°C. Remaining materials should be retained in the supplied container and sealed for future use.
Stability	Particles coupled by the user should be assessed for individual use and storage stability conditions, as this can vary depending on the protein and conditions used.
Applications	Particles are suitable for a range of fluorescent and chemiluminescent immunoassays and purification applications using molecules including proteins, peptides, antigens, and antibodies.
Supplied Surface	Particle: 1 µm, Superparamagnetic, 40% Magnetite. Concentration: 10 mg/mL (1% w/v solids).
Additives	Product contains ProClin® 300 as preservative.
Regulatory	For laboratory use only.

Compatibility	
Buffers	It is recommended to use the included buffers as the presence of certain materials will interfere with and reduce the coupling efficiency and storage stability. Mix&Go products are not compatible with high concentrations of Phosphates. For coupling, it is important to dilute the phosphate concentration, with the provided Coupling Buffer, to ≤ 2.5 mM (Antibody) and ≤ 0.1 mM (Antigens) as per the Chemical Compatibility Table on page 4.
Tubes	It is recommended to use low binding polypropylene microcentrifuge tubes.
Temperature	Do not freeze or expose to temperatures exceeding 60ºC. Room temperature is defined as 20ºC - 25ºC.
Protein Concentration	Protein coupling concentration is best optimised as this can vary depending on the protein used. The recommended concentration range for coupling antibody is 25 - 100 µg/mg particles, with a determined maximal binding capacity up to 30 µg/mg particles.
Magnet	When separating magnetic particles from supernatant, it is recommended to put the tube containing the particles on the magnetic separator until the supernatant is clear (up to 5 minutes), and carefully remove the supernatant so as not to disturb the particle pellet. Low strength magnets may take longer to form a pellet or may leave residue on the tube.

Safety
Standard safety precautions exercised when handling laboratory reagents should be adhered to. Refer to the product MSDS for safety precautions.

Warnings/Hazards
Product is not guaranteed DNase, RNase or endotoxin free. The user must determine the suitability of the product for specific uses.

Other Products
For more information on Anteo products please visit www.anteotech.com.

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Procedure		Time
Coupling Preparation		
1.	Allow all components to come to room temperature	
2.	Vortex particles	10 seconds
3.	Sonicate particles	1 minute
4.	Aliquot 100 µL (1 mg) of Activated 1 µm Magnetic Particles (Cat No. A-CMPAPMP) to a provided 1.5 mL tube	
Washing with Coupling Buffer		
5.	Pulse in a microcentrifuge to ensure particles are at the bottom of the tube before separation	
6.	Place the tube on the magnetic separator to separate particles from solution	1 minute
7.	Remove and discard the supernatant from the tube and then remove the tube from the magnet	
8.	Resuspend the particles in 100 µL of Coupling Buffer (Cat. No. A-CMPCBA1)	
9.	Vortex particles	10 seconds
ⓘ	Particles may aggregate and appear to stick on the tube during coupling. This may be part of the normal process whereby the surface during coupling is interacting with your protein of interest. Particles can be dispersed by sonication in a bath sonicator.	
10.	Repeat washing steps for a total of 2 washes	
Coupling		
11.	Pulse in a microcentrifuge to ensure particles are at the bottom of the tube before separation	
12.	Place the tube on the magnetic separator to separate particles from solution	1 minute
13.	Remove and discard the supernatant from the tube and then remove the tube from the magnet	
14.	Resuspend the particles in 50 µL of Coupling Buffer (Cat. No. A-CMPCBA1)	
15.	Vortex particles	10 seconds
16.	Prepare 50 µL of antibody at the required concentration in Coupling Buffer (Cat. No. A-CMPCBA1) in a fresh tube (provided)	
17.	Aspirate all of the prepared particles	
18.	Add the particles to the antibody solution	
19.	Vortex particles	10 seconds
ⓘ	Total volume is 100 µL total volume and particles are at 10 mg/mL.	
20.	Incubate at room temperature on a tube rotator to keep the particles in suspension	60 minutes
Blocking		
21.	Vortex particles	10 seconds
22.	Add 10 µL of the Blocking Buffer (Cat. No. A-CMPBBA1) to the tube	
23.	Vortex particles	10 seconds
24.	Incubate at room temperature on a tube rotator to keep the particles in suspension	60 minutes
Washing with Storage Buffer		
25.	Vortex particles	10 seconds
26.	Pulse in a microcentrifuge to ensure particles are at the bottom of the tube before separation	
27.	Place the tube on the magnetic separator to separate particles from solution	1 minute
28.	Remove and discard the supernatant from the tube and then remove the tube from the magnet	
29.	Resuspend the particles in 100 µL of Storage Buffer (Cat. No. A-CMPSBA1)	
30.	Vortex particles	10 seconds
31.	Repeat washing steps for a total of 2 washes	
Storage		
32.	Pulse in a microcentrifuge to ensure particles are at the bottom of the tube before separation	
33.	Place the tube on the magnetic separator to separate particles from solution	1 minute
34.	Remove and discard the supernatant from the tube and then remove the tube from the magnet	
35.	Resuspend the particles in 100 µL of Storage Buffer (Cat. No. A-CMPSBA1)	
36.	Vortex particles	10 seconds
ⓘ	Particles may be used immediately or stored at 2°C - 8ºC. Final particle concentration is 10 mg/mL.	

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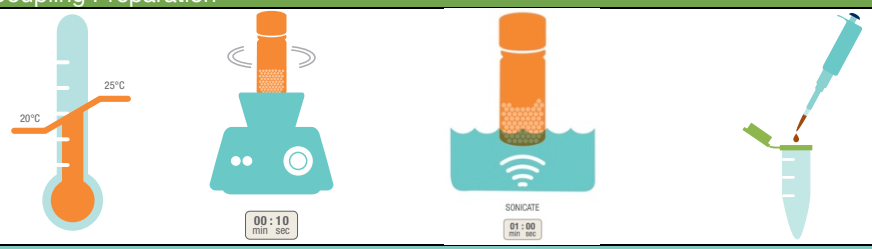
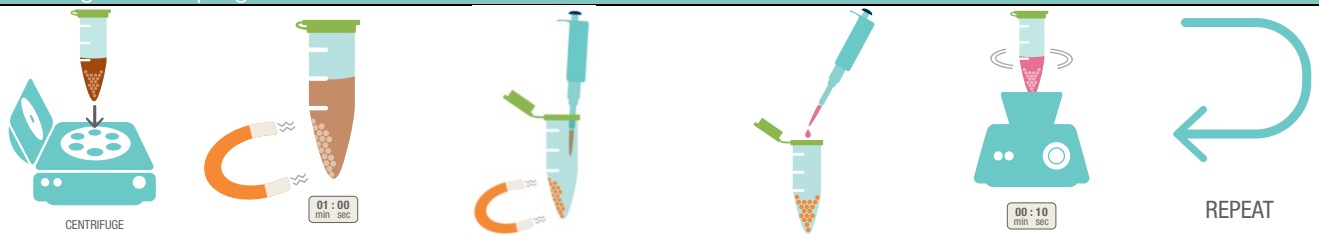
Procedure

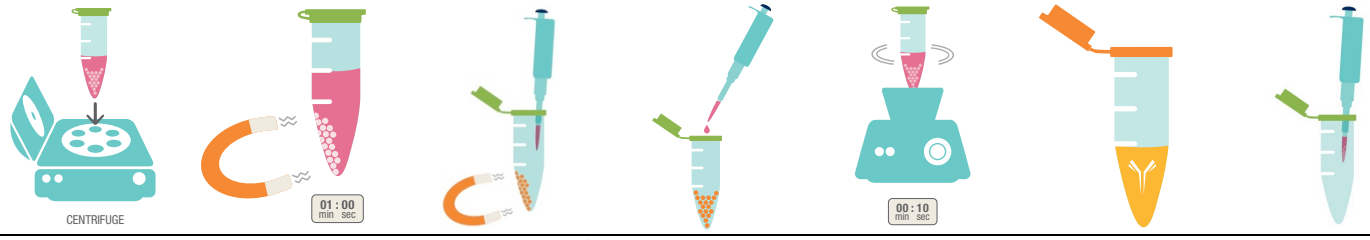
Coupling Preparation

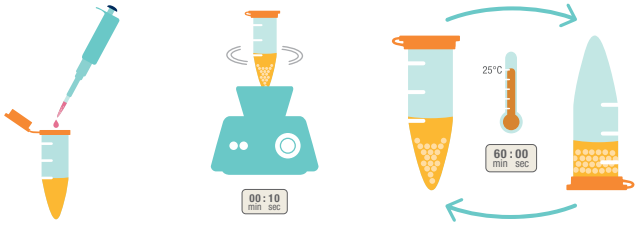


Washing with Coupling Buffer



Coupling

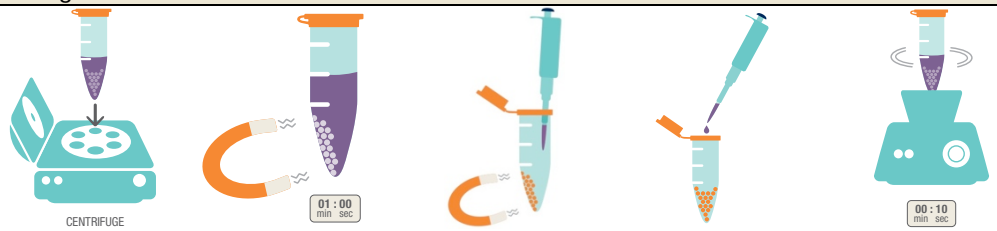




Blocking

Washing with Storage Buffer

Storage



AMG Coupling Kit, 1 µm Magnetic Particles

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Observations	
The inter-assay CV and intra-assay CV is assessed for every batch of particles manufactured, with a CV% of < 15% achieved for all batches. The monodispersity of particles refers to the level of aggregation. As particles aggregate, the percent monodispersity decreases. Activated particles are assessed by microscopy to determine the level of monodispersed particles.	
Binding capacity (Mouse IgG)	> 20 µg/mg particles
Monodispersity	> 90%

Chemical Compatibility Table

	Coupling	Storage	Assay
EDTA	✗	✗	≤ 10 mM
Phosphates (PBS 10 mM Phosphates, 150 mM NaCl)	≤ 2.5 mM (Antibody) ≤ 0.1 mM (Antigens)	✗	≤ 20 mM
Tween20®	≤ 0.5%	✗	≤ 0.5%
DMSO	≤ 25%	✗	≤ 25%
pH	Use Coupling Buffer (pH 5.2) provided	Use Storage Buffer provided	ND
Urea	≤ 4 M	✗	≤ 4 M
Saline	≤ 150 mM	≤ 150 mM	≤ 150 mM
Sodium Azide	≤ 0.1%	≤ 0.1%	≤ 0.1%
ProClin 300®	≤ 0.1%	≤ 0.1%	≤ 0.1%
Other Proteins	Co-coupling	✗	OK

Final concentration of chemicals during coupling

Contact

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