AMG™ Activation Kit for Multiplex Microspheres

AI-LMPAKMM-04.00

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

Materials Supplied	
Cat. No.	Component
A-CMPARA1	Mix&Go™ Activation Reagent
A-CMPCBB1	Coupling Buffer
A-CMPSBB1	Storage Buffer
n/a	1.5 mL microcentrifuge tubes

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

Specifications	
Ordering Information	A-LMPAKMM-10 (10 Reactions) A-LMPAKMM-40 (40 Reactions)
Storage	Store at 2°C - 8°C. Remaining materials should be retained in the supplied container and sealed for future use.
Stability	Microspheres 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	Compatible with multiplex microspheres such as Luminex® MagPlex® and MicroPlex® microspheres.
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.
Blocking	If blocking is required, perform optional blocking steps (step 23a – 23m) after coupling (step 23). BSA at 0.1% (w/v) diluted in Coupling Buffer may be used as a blocker.
Temperature	Do not freeze or expose to temperatures exceeding 60ºC. Room temperature is defined as 20ºC - 25ºC.
Protein Concentration	The recommended starting concentration for coupling antibody is 25 µg/mL. Protein coupling concentration is best optimised as this can vary depending on the protein used.
Magnet	When separating magnetic microspheres from supernatant, it is recommended to put the tube containing the microspheres 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.
Microspheres	This procedure is for magnetic MagPlex® microspheres. It is also compatible with MicroPlex® non-magnetic microspheres. To separate MicroPlex® microspheres use a centrifuge at 14,000 x g for 5 minutes.

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
Activation Preparation		
1.	Allow all components to come to room temperature	
2.	Resuspend microspheres by gentle inversion	1 minute
3.	Aliquot 100 µL of the microsphere suspension to a provided 1.5 mL tube	
4.	Place the tube on the magnetic separator to separate microspheres from solution	1 minute
5.	Remove and discard the supernatant from the tube and then remove the tube from the magnet	
Activation		
6.	Resuspend the microspheres in 100 µL of Mix&Go Activation Reagent (Cat. No. A-CMPARA1)	
7.	Vortex microspheres	10 seconds
8.	Incubate at room temperature on a tube rotator to keep the microspheres in suspension	60 minutes
ⓘ	Activated microspheres may be stored after this step at 2°C - 8°C for up to one year.	
Coupling Preparation		
9.	Vortex microspheres	10 seconds
10.	Sonicate microspheres	1 minute
Washing with Coupling Buffer		
11.	Pulse in a microcentrifuge to ensure microspheres are at the bottom of the tube before separation	
12.	Place the tube on the magnetic separator to separate microspheres from solution	1 minute
13.	Remove and discard the supernatant from the tube and then remove the tube from the magnet	
14.	Resuspend the microspheres in 100 µL of Coupling Buffer (Cat. No. A-CMPCBB1)	
15.	Vortex microspheres	10 seconds
16.	Repeat washing steps for a total of 2 washes	
Coupling		
17.	Prepare 100 µL of antibody at the required concentration in Coupling Buffer (Cat. No. A-CMPCBB1) in a fresh tube (not provided)	
18.	Pulse in a microcentrifuge to ensure microspheres are at the bottom of the tube before separation	
19.	Place the tube on the magnetic separator to separate microspheres from solution	1 minute
20.	Remove and discard the supernatant from the tube and then remove the tube from the magnet	
21.	Resuspend the microspheres in 100 µL of prepared antibody solution	
22.	Vortex microspheres	10 seconds
23.	Incubate at room temperature on a tube rotator to keep the microspheres in suspension	60 minutes
Optional Blocking		
ⓘ	If blocking is required, perform optional blocking steps (step 23a – 23m below).	
Washing with Storage Buffer		
24.	Vortex microspheres	10 seconds
25.	Pulse in a microcentrifuge to ensure microspheres are at the bottom of the tube before separation	
26.	Place the tube on the magnetic separator to separate microspheres from solution	1 minute
27.	Remove and discard the supernatant from the tube and then remove the tube from the magnet	
28.	Resuspend the microspheres in 100 µL of Storage Buffer (Cat. No. A-CMPSBB1)	
29.	Vortex microspheres	10 seconds
30.	Repeat washing steps for a total of 2 washes	
Storage		
31.	Pulse in a microcentrifuge to ensure microspheres are at the bottom of the tube before separation	
32.	Place the tube on the magnetic separator to separate microspheres from solution	1 minute
33.	Remove and discard the supernatant from the tube and then remove the tube from the magnet	
34.	Resuspend the microspheres in 100 µL of Storage Buffer (Cat. No. A-CMPSBB1)	
ⓘ	Store coupled microspheres at 2°C - 8°C.	

Optional Blocking		
Washing with Coupling Buffer		
23a.	Pulse in a microcentrifuge to ensure microspheres are at the bottom of the tube before separation	
23b.	Place the tube on the magnetic separator to separate microspheres from solution	1 minute
23c.	Remove and discard the supernatant from the tube and then remove the tube from the magnet	
23d.	Resuspend the microspheres in 100 µL of Coupling Buffer (Cat. No. A-CMPCBB1)	
23e.	Vortex microspheres	10 seconds
23f.	Repeat washing steps for a total of 2 washes	
Blocking		
23g.	Prepare 100 µL of blocker in Coupling Buffer (Cat. No. A-CMPCBB1) in a fresh tube (not provided)	
23h.	Pulse in a microcentrifuge to ensure microspheres are at the bottom of the tube before separation	
23i.	Place the tube on the magnetic separator to separate microspheres from solution	1 minute
23j.	Remove and discard the supernatant from the tube and then remove the tube from the magnet	
23k.	Resuspend the microspheres in 100 µL of blocker	
23l.	Vortex microspheres	10 seconds
23m.	Incubate at room temperature on a tube rotator to keep the microspheres in suspension	60 minutes

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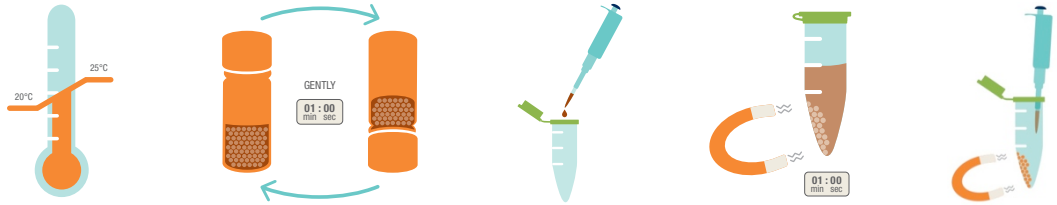
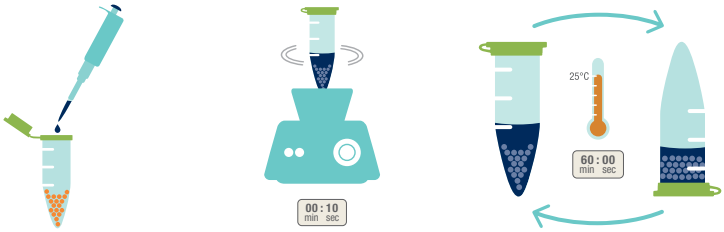
Procedure

Activation Preparation

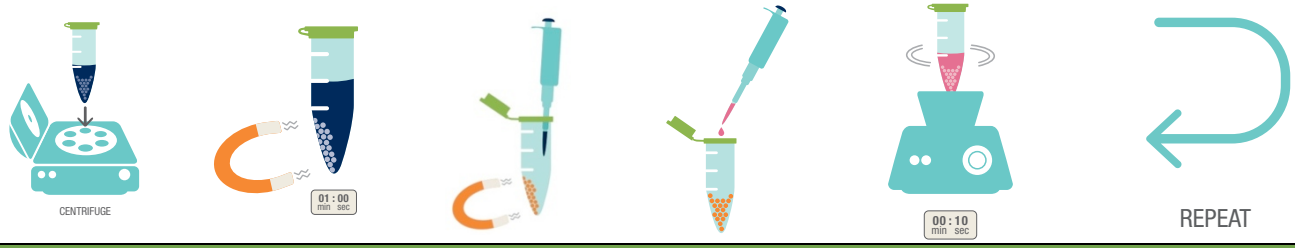


Activation

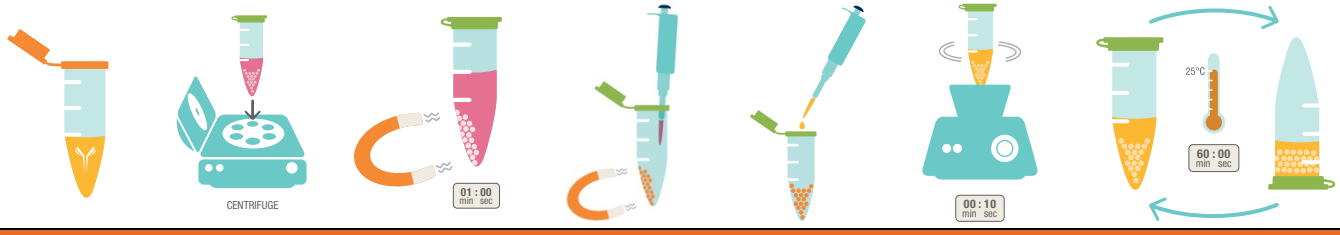


Coupling Preparation

Washing with Coupling Buffer



Coupling

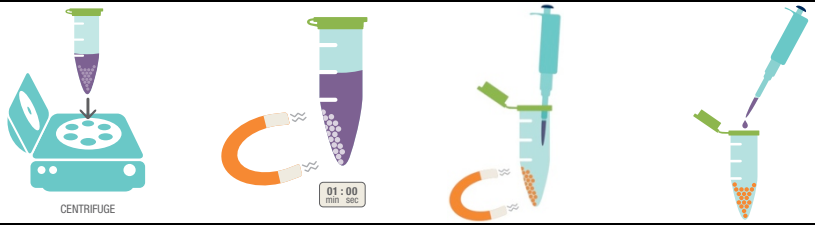


Optional Blocking

ⓘ | If blocking is required, perform optional blocking steps on the next page.

Washing with Storage Buffer

Storage



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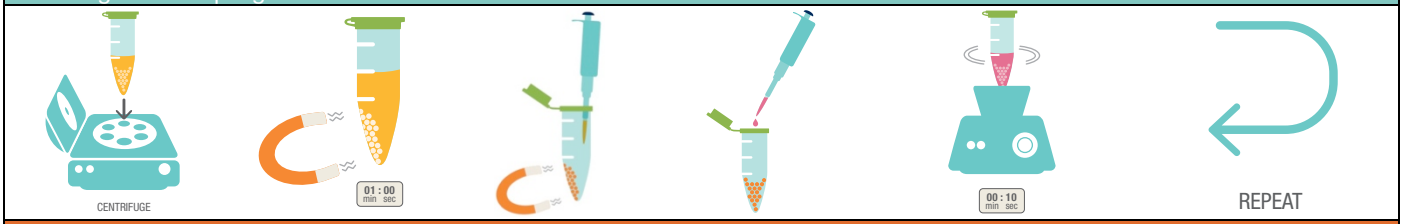
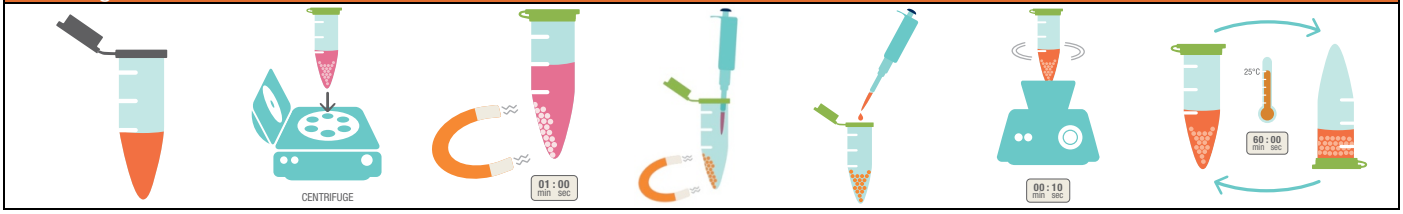
Optional Blocking

Washing with Coupling Buffer



Blocking



Chemical Compatibility Table

	Activation	Coupling	Storage	Assay
EDTA	✗	≤ 50 mM	✗	≤ 10 mM
Phosphates (PBS 10 mM Phosphates, 150 mM NaCl)	✗	≤ 2.5 mM (Antibody) ≤ 0.1 mM (Antigens)	✗	≤ 20 mM
Tween20®	✗	✗	✗	≤ 0.05%
DMSO	✗	≤ 40%	✗	ND
pH	Use Activation Reagent supplied	Use Coupling Buffer supplied	Use Storage Buffer supplied	ND
Urea	✗	≤ 4 M*	✗	ND
Saline	✗	≤ 1 M	≤ 150 mM	≤ 150 mM
Sodium Azide	✗	≤ 1%	≤ 0.1%	≤ 0.1%
ProClin 300®	≤ 0.05%	≤ 0.1%	≤ 0.1%	≤ 0.1%
Other Proteins	✗	Co-coupling	✗	OK
Carbohydrates	✗	≤ 50%	Use Storage Buffer supplied	ND
Cryoprotectant (Glycerol)	✗	< 50%	Use Storage Buffer supplied	ND
Temperature	20°C - 37°C	20°C - 37°C	2°C - 8°C	20°C - 37°C

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