

# AMG™ Coupling Kit, 200 nm Magnetic Particles

AI-LNPCKMP-07.00

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-CMPLNMP          | Activated 200 nm Magnetic Particles |
| A-CMPCBC1          | Coupling Buffer                     |
| A-CMPBBB1          | Blocking Buffer                     |
| A-CMPSBA1          | Storage Buffer                      |
| n/a                | 1.5 mL microcentrifuge tubes        |

| Additional Materials Required  |
|--|
| Protein of interest  |
| Pipettes   |
| Magnetic separator for tubes (capable to separate 200 nm magnetic particles) |
| Tube rotator   |
| Vortex mixer   |
| Microcentrifuge for 1.5 mL tubes   |
| Sonication bath with fresh deionised water (minimum power 60W)               |

| Specifications       |  |
|----------------------|--|
| Ordering Information | A-LNPCKMP-10 (10 Reactions)<br>A-LNPCKMP-30 (30 Reactions)   |
| Storage              | Store at 2°C - 8°C.<br>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 compatible with the majority of existing uses and protocols. This allows for easy substitution of the AMG™ Coupling Kit into your application of choice. |
| Supplied Surface     | Particle: 200 nm, Superparamagnetic, 70% Magnetite.<br>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 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 60°C.<br>Room temperature is defined as 20°C - 25°C.   |
| 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 100 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. |

| Other Products   |
|--|
| For more information on Anteo products please visit <a href="http://www.anteotech.com">www.anteotech.com</a> . |

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

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| Procedure   | Time       |
|---|------------|
| <b>Coupling Preparation</b>   |            |
| 1. Allow all components to come to room temperature   |            |
| 2. Vortex particles   | 10 seconds |
| 3. Sonicate particles   | 5 minutes  |
| 4. Aliquot 100 µL (1 mg) of Activated 200 nm Magnetic Particles (Cat No. A-CMPLNMP) to a provided 1.5 mL tube   |            |
| <b>Washing with Coupling Buffer</b>   |            |
| 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   | 5 minutes  |
| 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-CMPCBC1)  |            |
| 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  |            |
| <b>Coupling</b>   |            |
| 11. Prepare 100 µL of antibody at the required final concentration in Coupling Buffer (Cat. No. A-CMPCBC1) in a fresh tube (provided)   |            |
| 12. Pulse the particle tube in a microcentrifuge to ensure particles are at the bottom of the tube before aspiration  |            |
| 13. Aspirate all of the prepared particles  |            |
| 14. Add the particles to the antibody solution  |            |
| 15. Vortex particles  | 10 seconds |
| 16. Sonicate particles  | 5 minutes  |
| ⓘ Total volume is 200 µL total volume and particles are at 5 mg/mL.   |            |
| 17. Incubate at room temperature on a tube rotator to keep the particles in suspension  | 60 minutes |
| <b>Blocking</b>   |            |
| 18. Vortex particles  | 10 seconds |
| 19. Add 20 µL of the Blocking Buffer (Cat. No. A-CMPBBB1) to the tube   |            |
| 20. Vortex particles  | 10 seconds |
| 21. Incubate at room temperature on a tube rotator to keep the particles in suspension  | 60 minutes |
| <b>Washing with Storage Buffer</b>  |            |
| 22. Vortex particles  | 10 seconds |
| 23. Pulse in a microcentrifuge to ensure particles are at the bottom of the tube before separation  |            |
| 24. Place the tube on the magnetic separator to separate particles from solution  | 5 minutes  |
| 25. Remove and discard the supernatant from the tube and then remove the tube from the magnet   |            |
| 26. Resuspend the particles in 200 µL of Storage Buffer (Cat. No. A-CMPSBA1)  |            |
| 27. Vortex particles  | 10 seconds |
| 28. Repeat washing steps for a total of 2 washes  |            |
| <b>Storage</b>  |            |
| 29. Pulse in a microcentrifuge to ensure particles are at the bottom of the tube before separation  |            |
| 30. Place the tube on the magnetic separator to separate particles from solution  | 5 minutes  |
| 31. Remove and discard the supernatant from the tube and then remove the tube from the magnet   |            |
| 32. Resuspend the particles in 100 µL of Storage Buffer (Cat. No. A-CMPSBA1)  |            |
| 33. Vortex particles  | 10 seconds |
| 34. Sonicate particles  | 5 minutes  |
| 35. Vortex particles  | 10 seconds |
| 36. Sonicate particles  | 5 minutes  |
| ⓘ Particles may be used immediately or stored at 2°C - 8°C. Final particle concentration is 10 mg/mL. Resuspend particles (step 33 - 36) prior to use.  |            |

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| Procedure                    |  |  |  |  |  |  |        |
|------------------------------|--|--|--|--|--|--|--------|
| Coupling Preparation         |  |  |  |  |  |  |        |
|                              |  |  |  |  |  |  |        |
| Washing with Coupling Buffer |  |  |  |  |  |  |        |
|                              |  |  |  |  |  |  | REPEAT |
| Coupling                     |  |  |  |  |  |  |        |
|                              |  |  |  |  |  |  |        |
| Blocking                     |  |  |  |  |  |  |        |
|                              |  |  |  |  |  |  |        |
| Washing with Storage Buffer  |  |  |  |  |  |  |        |
|                              |  |  |  |  |  |  | REPEAT |
| Storage                      |  |  |  |  |  |  |        |
|                              |  |  |  |  |  |  |        |

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| Chemical Compatibility Table                      |  |                                |          |
|---|--|--------------------------------|----------|
|   | Coupling                                 | Storage                        | Assay    |
| EDTA  | <b>X</b>                                 | <b>X</b>                       | ≤ 10 mM  |
| Phosphates<br>(PBS 10 mM Phosphates, 150 mM NaCl) | ≤ 2.5 mM                                 | <b>X</b>                       | ≤ 20 mM  |
| Tween20®  | ≤ 0.25%                                  | <b>X</b>                       | ≤ 0.5%   |
| DMSO  | ≤ 15%                                    | <b>X</b>                       | ≤ 30%    |
| pH  | Use Coupling Buffer<br>(pH 5.2) provided | Use Storage Buffer<br>provided | ND       |
| Urea  | ≤ 2 M                                    | <b>X</b>                       | ≤ 2 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                              | <b>X</b>                       | OK       |

Final concentration of chemicals during coupling.

| Contact  |                            |                         |
|--|----------------------------|-------------------------|
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