AnteoTech

AnteoBind™ Sub-Micron

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RUO For research use only.
Not for use in diagnostic procedures

Symbol Glossary

The following symbols can be found on kit packaging and components and throughout this instruction for use:

Symbol	Meaning	Symbol	Meaning
	Identifies the catalogue number.		Identifies the batch or lot code.
	Identifies the manufacturer of the kit.		Indicates the maximum and minimum storage temperature limits.
	Indicates the volume of the kit component.		Indicates the kit expiration date.
	Indicates that the kit is for research use only.		Indicates that the instructions for use shall be consulted.
	**GHS08 – Health Hazard:** Chronic health hazards; this may include aspiratory and respiratory hazards, carcinogenicity, mutagenicity, and reproductive toxicity.		**GHS05 – Corrosion:** Corrosive chemicals, may cause severe skin and eye damage and may be corrosive to metals.
	**GHS07 – Exclamation mark:** Low level toxicity. This includes respiratory, skin, and eye irritation, skin sensitisers and chemicals harmful if swallowed, inhaled or in contact with skin.		

Warnings and Precautions

1. The Instruction for Use (IFU) must be read and understood prior to commencing the use of this kit.
2. For research use only. Not for use in diagnostics procedures.
3. Safety Data Sheet (SDS) is available by contacting AnteoTech Technical Support.
4. Wear appropriate personal protective equipment when using this kit.
5. Follow institutional safety procedures for working with chemicals and handling biological samples.
6. Handle waste as per institutional procedures and in accordance with local regulations.
7. Do not use the kit beyond the expiration date.

AnteoTech Technical Support

For assistance and support please contact AnteoTech Technical Support for guidance.

Telephone: +61 7 3219 0085

Email: support@anteotech.com

For additional information, visit our website www.anteotech.com

This IFU may be updated periodically. To ensure that you have the current version, please visit https://www.anteotech.com/life-science/products/ or contact AnteoTech Technical Support.

Description

AnteoBind™ Sub-Micron was developed to activate carboxylated particles 0.5 to 1 µm in diameter in preparation for biomolecule conjugation. AnteoBind™ is a molecular glue comprised of polymeric metal ions that facilitates conjugation via the utilisation of co-ordination avidity binding of synthetic surfaces and biomolecules. The result is a simplified conjugation process that provides secure biomolecule binding in native conformations.

AnteoBind™ Sub-Micron is available in 5 mL (activates 50 mg of particles), 10 mL (activates 100 mg of particles), and custom volume configurations that can be used to perform activations at scales ranging from 1 to 100 mg of particles per reaction.

Due to the vast diversity of biomolecule composition, conjugation performance is not guaranteed and must be optimised by the end user. For assistance and support regarding biomolecule conjugation please contact AnteoTech Technical Support.

Principles of AnteoBind™

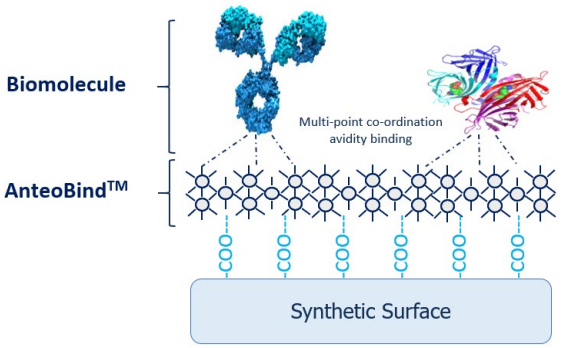


Image left: Schematic representation of AnteoBind functioning as a molecular glue, facilitating the conjugation of synthetic surfaces and biomolecules.

The conjugation process involves two major steps, particle activation with AnteoBind and AnteoBind enabled biomolecule conjugation. The AnteoBind™ technology takes advantage of supramolecular chemistry, that is, the generation of non-covalent bonds between molecules. AnteoBind contains a proprietary water based oligomeric metal-ion complex that creates a nanometre thin molecular glue on the particle surface, in essence 'activating' the particle surface, priming it for secure biomolecule binding in native conformations.

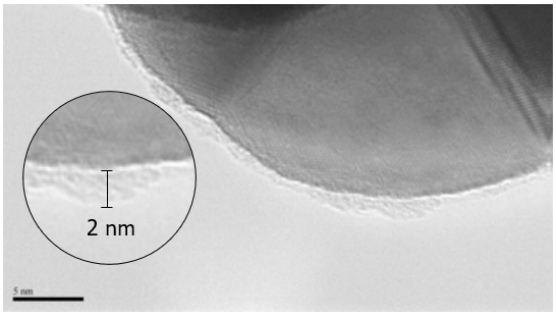


Image left: Transmission electron microscope image demonstrating surface activation. This image of an activated gold nanoparticle demonstrates that the surface is coated in approximately 2 nm of AnteoBind and is ready for biomolecule conjugation.

AnteoBind activated particles have been demonstrated to remain stable at 2-8 °C for 1 year. This kit allows the user to either activate particles in bulk or multiple small lots using the same reagents, providing the user with enhanced experimental reproducibility. This differs from EDC chemistry where the components must be used immediately after reconstitution.

Provided Materials

Component	Reference	Amount provided		
		5 mL	10 mL	Custom
AnteoBind™ Sub-Micron	A-SMPN100	1 x 5 mL	1 x 10 mL	Variable

Required Materials – not provided

- Carboxylated particles 0.5 to 1 µm in diameter
- Low binding polypropylene reaction tubes
- Low binding micropipette tips
- Conjugation Buffer and biomolecule
- Blocking Buffer and blocker agent
- Storage Buffer

Suggested Equipment

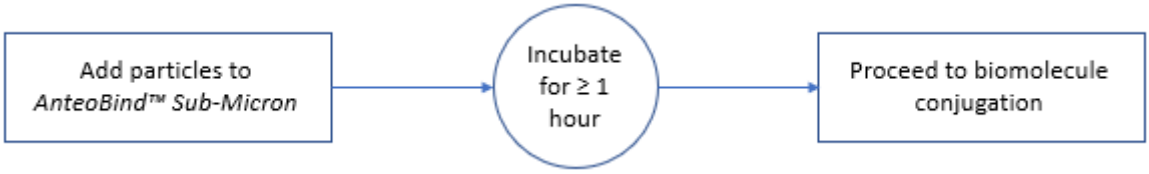
Process	Equipment required
Particle dispersion	Ultrasonicator (liquid or solid medium)
Separation of non-magnetic particles	Centrifuge
Separation of magnetic particles	Magnetic tube rack
Solution and buffer transfer/supernatant removal	Micropipettes
Particle incubation	Tube rotator, roller, or mixer
Sample mixing	Vortex mixer
Sample spot centrifugation	Microcentrifuge

Special Operating Instructions

Particle compatibility	Particles must be pre-functionalised with carboxyl (-COOH) functional groups. Particles must be activated and conjugated at 10 mg/mL.
Biomolecule compatibility	Not compatible with short peptides or small molecules. Biomolecule compatibility is not guaranteed and must be determined by the user.
Particle separation	Magnetic particles: magnetic separator required. Separator parameters should be optimised for specific particle types. Non-magnetic particles: centrifugation required. Centrifugation parameters should be optimised for specific particle types.
Particle aggregation	Pipette-mix, vortex-mix and/or ultrasonicate particles until dispersed. Ultrasonication parameters must be optimised for particle type.
Scale	This kit has been tested at 1 to 100 mg particle amounts.

Optional particle analysis	It is recommended that particle diameter, polydispersity index and zetapotential is determined prior to and after *Step 1: Particle Activation.* Particle diameter, polydispersity index may be determined via Dynamic Light Scattering analysis and zetapotential may be determined via Laser Doppler Electrophoresis with the following expected characteristics: • Raw material: low polydispersity index value, negative zetapotential. • Activated particles: larger than raw material, low polydispersity index value, positive zetapotential. The generated data will allow the user to establish particle compatibility and to determine the effectiveness of the activation process.

Process Workflow



General Procedure

The following procedure details the general process for the activation of 5 mg of carboxylated particles 0.5 to 1 µm in diameter with AnteoBind.

Refer to Special Operating Instructions above prior to the commencement of this procedure.

Before commencing please note:

- **Particles must be pre-functionalised with carboxyl (-COOH) functional groups.**
- **Particles must be adjusted to 100 mg/mL prior to activation.**
- Ensure all materials are at room temperature before use.
- Use a micropipette to remove supernatant taking care not to disturb the particle pellet.
- Vortex-mix particles prior to use. Ultrasonicate if required.
- It is recommended that particle diameter, polydispersity index and zetapotential is determined prior to and after *Step 1: Particle Activation.*

Step 1: Particle Activation

1. Transfer 450 µL of *Particle Activation Solution* to a new reaction tube.
2. Transfer 50 µL (5 mg) of particles to the reaction tube.
3. Vortex-mix and then ultrasonicate until dispersed.
4. Incubate for a minimum of 1 hour at room temperature under continuous gentle agitation (e.g. tube rotator at 25 rpm).

Note: *The optimal incubation period may be determined by the user.*

Note: *Activated particles are at 10 mg/mL and are ready for immediate conjugation. Alternatively, activated particles may be stored at 2-8 °C for 1 year.*

Trouble Shooting

Issue	Possible Cause(s)	Recommendations
Aggregation	Insufficient sample mixing	Always vortex mix samples for at least 10 seconds.
	Particles not appropriately dispersed	Optimise ultrasonication parameters. AnteoTech recommends liquid medium ultrasonication at 384W or solid medium ultrasonication at 12W.
	Particles are unstable	Contact AnteoTech Technical Support.
Loss of particles	Inappropriate centrifugal separation	Use appropriate centrifuge settings and rotors for the particle type and sample volumes being processed. Contact AnteoTech Technical Support for further details.
	Inappropriate magnetic separation	Ensure that the magnetic separator is appropriate for the particle type and sample volume being processed. Limit the exposure of particles to magnetic forces. Overexposure may induce permanent magnetisation and irreversible aggregation of particles. Contact AnteoTech Technical Support for further details.
	Pipetting	Take care not to disturb the particle pellet during supernatant removal. Do not directly aim the pipette tip at the pellet or excessively agitate the supernatant.
	Prolonged storage	Suspend particles thoroughly before use. Vortex mix for at least 10 seconds followed by ultrasonication. Contact AnteoTech Technical Support for further details.