AnteoTech

AnteoBind™ NXT

(For Nano and Microparticles)

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

Symbol Glossary

The following symbols can be found on reagent 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 reagent.		Indicates the maximum and minimum storage temperature limits.
	Indicates the volume of the reagent component.		Indicates the reagent expiration date.
	Indicates that the reagent is for research use only.		Indicates that the instructions for use shall be consulted.

Warnings and Precautions

1. The Instruction for Use (IFU) must be read and understood prior to commencing the use of this reagent.
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 reagent.
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. The use of low-binding tubing and micropipette tips is highly recommended to minimise particle loss.
8. Do not use the reagent 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™ 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™ NXT is available in 5 mL (activates up to 50 mg of particles), 50 mL, 100 mL, 250 mL and 1 L volume configurations that can be used to perform the activation of carboxylated particles nanoparticles ≥ 150 nm and microparticles ≥ 1 µm in diameter at scales ranging from 1 to 10 mg of particles per reaction. The ability to activate particles in bulk for storage and subsequent use allows users developing novel assays to perform numerous small-scale conjugations to test multiple conjugation and blocking parameters. The conjugated particles can then be used in particle-based assays, such as, but not limited to chemiluminescent assays, bioseparations or for lateral flow assay development purposes.

Due to the vast diversity of biomolecule composition, conjugation performance may vary and requires biomolecule specific optimisations by the end user. For assistance and support regarding biomolecule conjugation please contact AnteoTech Technical Support (support@anteotech.com).

Principles of AnteoBind™

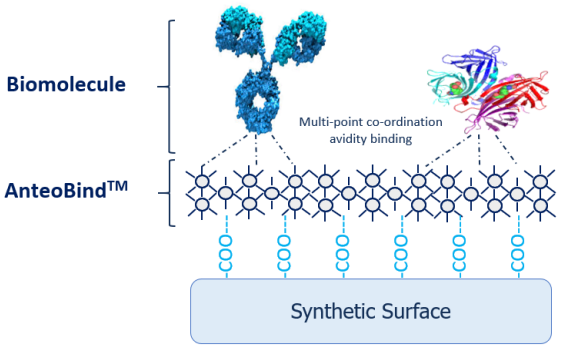


Image left: Schematic representation of AnteoBind™ functioning as a molecular glue, facilitating the conjugation of synthetic surfaces and biomolecules.

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 proprietary water-based oligomeric metal-ion complexes that create a nanometer thin molecular glue on the particle surface, in essence 'activating' the particle surface, priming it for secure biomolecule binding in native conformations.

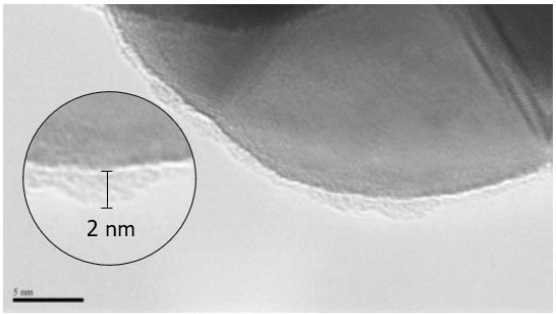


Image left: Transmission electron microscope image demonstrating surface activation. This image of an activated gold nanoparticle demonstrates that the surface is coated in approximately 2 nm of AnteoBind™ and is ready for biomolecule conjugation.

AnteoBind™ activated particles have been demonstrated to remain stable at 2-8 °C for 1 year. AnteoBind™ users have the flexibility to activate particles in bulk or in multiple smaller lots using the same reagents. This feature enhances experimental reproducibility and offers a notable advantage over conventional EDC chemistry-based activation and conjugation methods. Unlike those methods, where the chemical components must be used immediately after reconstitution, the AnteoBind™ reagent allows for extended usage and eliminates the need for immediate utilisation.

Provided Materials

Component	Reference	Amount provided	Other information
AnteoBind™ NXT	A-LNPNXT	1 x 5 mL	Also available in bulk volumes
Particle Wash Solution	A-CMPWSF1	1 x 15 mL	Can be substituted with ddH_2O
Conjugation Buffer	A-CMPCBF1	3 X 15 mL	25 mM MES pH 6.0 + 0.05% ProClin™ 300
Blocker Diluent	A-CMPBDF1	1 x 15 mL	Can be substituted with Conjugation Buffer
Storage Buffer	A-CMPSBF1	2 x 15 mL	50 mM TBS pH 8.0 + 0.05% sodium azide

Required Materials – not provided*

- Carboxylated particles ≥ 150 nm or ≥ 1 µm in diameter
- Low binding polypropylene reaction tubes **- Highly Recommended**
- Low binding micropipette tips **- Highly Recommended**
- Biomolecule prepared in *Conjugation Buffer*
- Blocking Agent prepared in *Blocker Diluent*

** **Note:** For the standalone AnteoBind™ NXT reagent, the user will have to prepare the additional recommended buffers (Particle Wash Solution, Conjugation Buffer, Blocker Diluent and 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

Biomolecule Compatibility	This AnteoBind™ formulation has been used to conjugate antibodies, antigens, Fab fragments, streptavidin, Protein A/G, fluorescent proteins, and oligonucleotides. Biomolecule compatibility may vary and must be determined by the user.
Particle Compatibility	Particles must be pre-functionalised with carboxyl (-COOH) functional groups.

Recommended particle concentrations for activation and conjugation:

Particle Size/Type	Activation (Particle concentration)	Conjugation (Particle concentration)
≥150 nm and <1 µm (latex)	10 mg/ml (up to)	5 mg/ml
≥1 µm	10 mg/ml (up to)	10 mg/ml
Gold nano shells/nanorods	60 OD/ml	60 OD/ml

Optimisation may be required based on the carboxyl load of the bare particle. Please contact AnteoTech Technical Support (support@anteotech.com) for further details. This AnteoBind™ formulation has been used to conjugate biomolecules to 300 nm Merck Estapor Latex Particles (K1-030), ThermoFisher Coloured Polystyrene Microspheres (#CDFDB1040CA and equivalent), Invitrogen™ Dynabeads™ M-270 Carboxylic Acid and MyOne™ Carboxylic Acid, Merck Estapor Encapsulated (EM1-100/40) and Magnetic Microspheres (M1-030/40), Europium particles (F1-Eu-030), Agilent LodeStars Magnetic Carboxyl (PL6727-0003), as well as nanoComposix 150 nm BioReady Carboxyl Gold Nanoshells.

Biomolecule Concentration

Protein: this reagent has been used at ranges of 2 to 150 µg of proteins per mg of particles.

Oligonucleotide: AnteoBind™ reagents have been used at 4,000 pmol of oligonucleotides per mg of particles.

Optimal biomolecule concentration may vary depending on particle, conjugate, and assay type and must be determined by the user.

Co-Conjugation

Unlike traditional chemistries one-step co-conjugation of two or more biomolecules is possible using AnteoBind™. This can reduce non-specific binding, reduce target biomolecule usage, or allow for multiple biomolecules to be bound to one particle at the same time. It is recommended as a starting point by pre-mixing antibody: spacer agent/co-conjugant at 1:1 mass ratio in *Conjugation Buffer* before use. This also applies to other co-conjugation options such as antibody + Blocking Agent, antibody + streptavidin, antibody 1 + antibody 2 etc. If you have questions, please contact AnteoTech Technical Support (support@anteotech.com) for further details.

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, however as a starting point use the recommended particle manufacturer instructions.

Particle Dispersion

Adequate dispersion of particles is crucial at each step, particularly in the case of non-magnetic particles that undergo centrifugation. If monodispersity is not achieved (refer to *Optional Particle Analysis* section) incomplete activation, conjugation or blocking of particles may occur, leading to particle aggregation and sub-optimal performance.

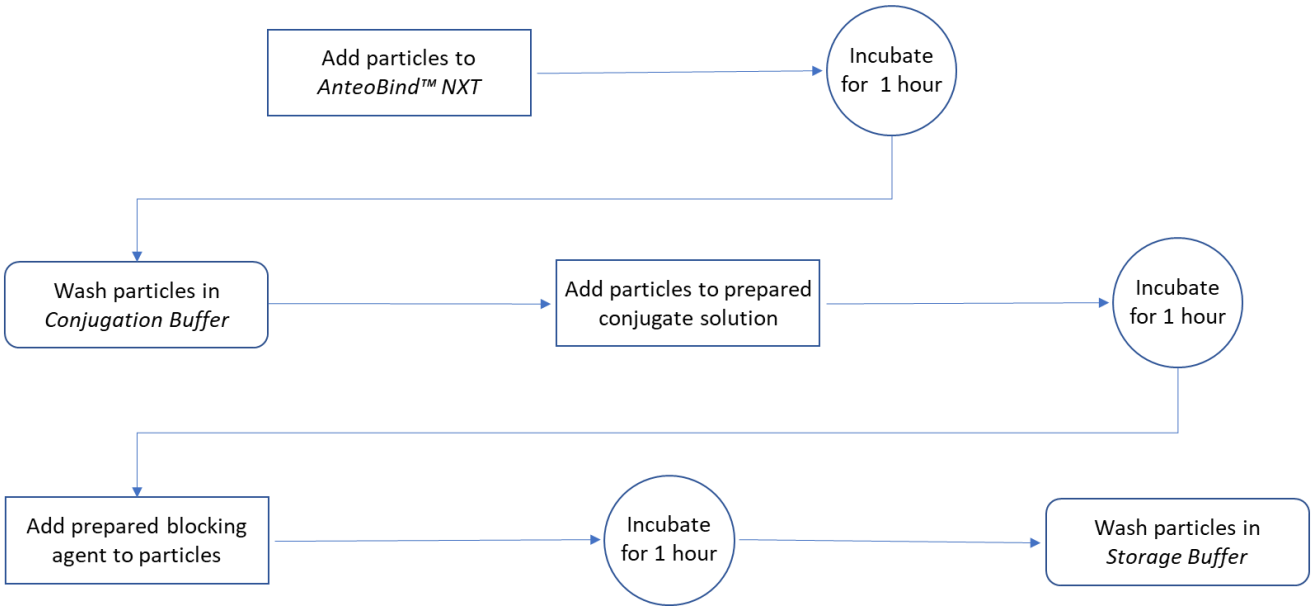
Pipette-mix, vortex-mix and/or ultrasonicate particles until dispersed. Ultrasonication parameters must be optimised for particle and conjugate types.

At smaller scales, indirect sonication methods are recommended during activation and wash steps, as over time AnteoBind™ may coat probes utilised in direct sonication methods. Alternatively separate probes can safely be used for AnteoBind™-related steps and post conjugation steps.

The sonication power utilised and length of time required to achieve complete particle dispersion will vary based on particle and solution characteristics and must be optimised by the user.

Particle Aggregation	AnteoTech recommends particle aggregation is assessed via a hemocytometer under compound microscopy prior to and after *Step 1: Particle Activation,* after *Step 2: Activated Particle Wash* and after *Step 5: Storage of Conjugated Particles*. Particles should appear > 90% monodisperse. Co-conjugation with a Blocking Agent (e.g. BSA) during conjugation, may inhibit conjugation induced aggregation. Co-conjugation parameters must be optimised for particle and conjugate types.
Blocker Preparation	This procedure recommends using the Conjugation Buffer or the supplied Blocker Diluent (if using a kit configuration) for Blocker Agent preparation. AnteoTech recommends the use of ≥ 98% pure, protease free bovine serum albumin (BSA) (Merck Product Number: A7030) at 10% (w/v) in *Conjugation Buffer.* The optimal Blocking Agent may vary between different particles, conjugates, and assay systems and must be optimised by the user.
Scale	This procedure has been successfully used to activate and conjugate particle batches ranging from 1 to 100 mg. For custom reagents to activate a specific amount of particles, please contact AnteoTech Technical Support (support@anteotech.com) for further details.
Conjugated Particle Storage and Stability	If not immediately used AnteoTech recommends storage of conjugated particles at 2-8 °C under continuous gentle agitation (e.g. tube rotator, mixer, or roller at 25 rpm). It is essential for the user to determine the stability of each conjugated particle type in the recommended *Storage Buffer*. The stability of the conjugated particles may vary depending on factors such as the nature of the particles, the biomolecule conjugated, additives used, and the intended application.
Optional Particle Analysis	AnteoTech recommends that particle diameter, polydispersity index and zetapotential is determined prior to and after *Step 1: Particle Activation* and after *Step 2: Activated Particle Wash* and after *Step 5: Storage of Conjugated Particles.* 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. • Conjugated particles: larger than activated material, low polydispersity index, shift towards negative zetapotential. The generated data will allow the user to establish particle compatibility and to determine the effectiveness of the activation process. ***Note:*** *High density particles (e.g. superparamagnetic particles) may not be suitable for Dynamic Light Scattering analysis. These particles may be assessed for aggregation via a hemocytometer under compound microscopy.* *In some instances, the observed size of the activated particles may be smaller compared to the starting material. This may be due to the presences of surfactants or other storage additives that impact the size measurement, or the recorded sizes may fall within the range of instrument error. A shift to a positive zetapotential confirms the successful activation of particles.*

Process Workflow



General Procedure — Nanoparticles

The following procedure details the general process for the activation of 5 mg of carboxylated particles ≥ 0.150 nm and < 1 µm in diameter with AnteoBind™ NXT. This procedure is applicable to both AnteoBind™ NXT kit and standalone AnteoBind™ NXT reagent.

Please familiarise yourself with the **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 at a concentration of 100 mg/mL prior to activation, if adjustment is required, set aside the required amount of particles, separate the particles from solution and remove the required amount of supernatant before resuspending.**
- **Particles supplied with surfactant in the stock solution should be washed once with Type 1 water prior to Step 1: Particle Activation and resuspended in Type 1 water to a final concentration of 100 mg/ml. If you are unsure of whether your particles contain surfactant, please conduct this pre-wash step.**
- 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.
- Where possible, it is recommended that particle diameter, polydispersity index and zetapotential is determined prior to and after *Step 1: Particle Activation* and after *Step 2: Activated Particle Wash* and after *Step 5: Storage of Conjugated Particles.*

Step 1: Particle Activation

1. Transfer 450 µL of ***AnteoBind™ NXT 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 homogenously. If using a water bath sonicator find the sonicator sweet spots to increase efficiency.

Note: *The water bath sweet spot is defined as the liquid surface area displaying the most disturbance, an indication of where the most energy is generated.*

4. Incubate for 1 hour at room temperature under continuous gentle agitation (e.g. tube rotator at 25 rpm).

Note: *Depending on the characteristics of the particles, the optimal incubation period may vary, and user-driven optimisations may be necessary. Factors such as the size, composition, and surface properties of the particles can influence the activation process.*

Activated particles are at 10 mg/mL and are ready for immediate conjugation. Alternatively, activated particles may be stored at 2-8 °C for 24 months.

Step 2: Activated Particle Wash

1. Separate the activated particles from solution as per particle manufacturer's instructions and remove at least 95% (475 µL) of the supernatant.
2. Reconstitute the pellet to the same final volume as in Step 1.3 (500 µL) by adding *Conjugation Buffer* to the particles, vortex mix and ultrasonicate until dispersed homogenously.
3. Repeat steps 1 and 2.
4. Separate the activated particles from solution and remove at least 95% (475 µL) of the supernatant.
5. Reconstitute the pellet to the final volume in step 2.2 (500 µL) by adding *Conjugation Buffer* to the particles, vortex mix and ultrasonicate until dispersed homogenously.

Step 3: Particle Conjugation

1. Prepare biomolecule to be conjugated at the required concentration in *500 µL* of Conjugation Buffer using the below table as a guide.

Target biomolecule to be conjugated per mg of particles (µg/mg)	**Biomolecule concentration prepared in 500 µL of *Conjugation Buffer* (µg/mL)**
5	50
25	250
50	500
100	1000

Note: *As a general rule, if the target biomolecule loading per mg of particles is **X** µg, the biomolecule should be prepared at 10**X** µg/mL in Conjugation Buffer.*

2. Add the entirety of the contents (≥ 500 µL) from the washed particle tube (Step 2.5) to the entirety of the prepared conjugate (Step 3.1). Vortex-mix until fully dispersed.
3. Incubate for 1 hour at room temperature under continuous gentle agitation.

Note: *Particle aggregation may occur once activated particles have been added to the prepared biomolecule. This occurs due to the uptake of biomolecule resulting in a neutral surface charge and is generally reversed during Step 4: Particle Blocking and Step 5: Storage of Conjugated Particles.*

Depending on the characteristics of the particles, the optimal incubation period may vary, and user-driven optimisations may be necessary. Factors such as the size, composition, and surface properties of the particles can influence the conjugation process.

Step 4: Particle Blocking

Note: *The optimal Blocking Agent may vary between different particles, biomolecules, and assay systems.*

AnteoTech recommends using Merck Product Number: A7030 BSA at 10% (w/v) prepared in Conjugation Buffer for antibody conjugations. Smaller molecular weight blockers at lower working concentrations are recommended when working with smaller molecular weight biomolecules (oligos, antigens etc.).

1. Prepare Blocker Agent at the required concentration in 150 µL of *Conjugation Buffer* ***(or Blocker Diluent if available)***.
2. Add 10% of the total volume of solution in Step 3.2 (100 µL) of prepared Blocking Agent directly to the particle reaction tube.
3. Vortex-mix and ultrasonicate until fully dispersed homogenously.
4. Incubate for 1 hour at room temperature under continuous gentle agitation.

Note: *Particle aggregation may occur. This may be reversed during Step 5: Storage of Conjugated Particles.*

Depending on the characteristics of the particles, the optimal incubation period may vary, and user-driven optimisations may be necessary.

Step 5: Storage of Conjugated Particles

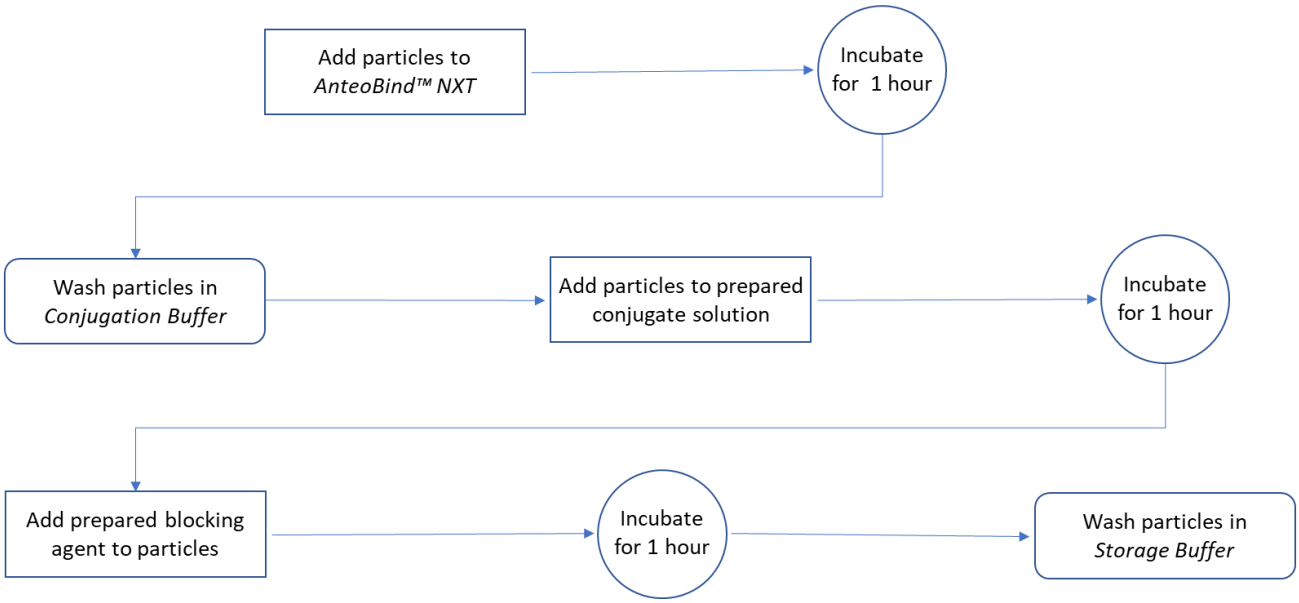
1. Separate the activated particles and remove at least 95% (1045 µL) of the supernatant.
2. Add 20 X the initial volume of particle in Step 1.2 (1000 µL) of *Storage Buffer* to the particles, vortex mix and ultrasonicate until dispersed homogenously.
3. Repeat steps 1 and 2 twice.
4. Separate the conjugated particles and remove at least 95% (1000 µL) of the supernatant.
5. Reconstitute the pellet to the final volume in Step 1.3 (500 µL) by adding *Storage Buffer* to the particles, vortex mix and ultrasonicate until dispersed homogenously.
6. The particles are approximately 10 mg/mL and are ready for use or may be stored at 2 to 8 °C until required.

Note: *The end user must determine the stability of each conjugate in Storage Buffer and whether additives may be required to stabilise the conjugated biomolecule.*

AnteoTech recommends using techniques such as dry weight measurements or absorbance scans to determine the overall particle concentration.

Thoroughly resuspend particles via vortex-mixing and sonication before use.

Process Workflow



General Procedure — Microparticles

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

Please familiarise yourself with the **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 at a concentration of 100 mg/mL prior to activation, if adjustment is required, set aside the required amount of particles, separate the particles from solution and remove the required amount of supernatant before resuspending.**
- **Particles supplied with surfactant in the stock solution should be washed once with Type 1 water prior to Step 1: Particle Activation and resuspended in Type 1 water to a final concentration of 100 mg/ml. If you are unsure of whether your particles contain surfactant please conduct this pre-wash step.**
- 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.
- Where possible, it is recommended that particle diameter, polydispersity index and zetapotential is determined prior to and after *Step 1: Particle Activation* and after *Step 2: Activated Particle Wash* and after *Step 5: Storage of Conjugated Particles.*

Step 1: Particle Activation

1. Transfer 450 µL of ***AnteoBind™ NXT 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 homogenously. If using a water bath sonicator find the sonicator sweet spots to increase efficiency.

Note: *The water bath sweet spot is defined as the liquid surface area displaying the most disturbance, an indication of where the most energy is generated.*

4. Incubate for 1 hour at room temperature under continuous gentle agitation (e.g. tube rotator at 25 rpm).

Note: *Depending on the characteristics of the particles, the optimal incubation period may vary, and user-driven optimisations may be necessary. Factors such as the size, composition, and surface properties of the particles can influence the activation process.*

Activated particles are at 10 mg/mL and are ready for immediate conjugation. Alternatively, activated particles may be stored at 2-8 °C for 24 months.

Step 2: Activated Particle Wash

1. Separate the activated particles from solution as per particle manufacturer's instructions and remove at least 95% (475 µL) of the supernatant.
2. Reconstitute the pellet to the same final volume as in Step 1.3 (500 µL) by adding *Conjugation Buffer* to the particles, vortex mix and ultrasonicate until dispersed homogenously.
3. Repeat steps 1 and 2.
4. Separate the activated particles from solution and remove at least 95% (475 µL) of the supernatant.
5. Reconstitute the pellet to 10% of the final volume in step 2.2 (50 µL) by adding *Conjugation Buffer* to the particles, vortex mix and ultrasonicate until dispersed homogenously.

Step 3: Particle Conjugation

1. Prepare biomolecule to be conjugated at the required concentration in *450 µL* of Conjugation Buffer using the below table as a guide.

Target biomolecule to be conjugated per mg of particles (µg/mg)	Biomolecule concentration prepared in 500 µL of *Conjugation Buffer* (µg/mL)
5	55.5
25	277.5
50	555
100	1110

Note: *As a general rule, if the target biomolecule loading per mg of particles is* ***X*** *µg, the biomolecule should be prepared at 11.1****X*** *µg/mL in Conjugation Buffer.*

2. Add the entirety of the contents (≥ 50 µL) from the washed particle tube (Step 2.5) to the entirety of the prepared conjugate (Step 3.1). Vortex-mix until fully dispersed.
3. Incubate for 1 hour at room temperature under continuous gentle agitation.

Note: *Particle aggregation may occur once activated particles have been added to the prepared biomolecule. This occurs due to the uptake of biomolecule resulting in a neutral surface charge and is generally reversed during Step 4: Particle Blocking and Step 5: Storage of Conjugated Particles.*

Depending on the characteristics of the particles, the optimal incubation period may vary, and user-driven optimisations may be necessary. Factors such as the size, composition, and surface properties of the particles can influence the conjugation process.

Step 4: Particle Blocking

Note: *The optimal Blocking Agent may vary between different particles, biomolecules, and assay systems.*

AnteoTech recommends using Merck Product Number: A7030 BSA at 10% (w/v) prepared in Conjugation Buffer for antibody conjugations. Smaller molecular weight blockers at lower working concentrations are recommended when working with smaller molecular weight biomolecules (oligos, antigens etc.).

1. Prepare Blocker Agent at the required concentration in 100 µL of *Conjugation Buffer* ***(or Blocker Diluent if available)***.
2. Add 10% of the total volume of solution in Step 3.2 (50 µL) of prepared Blocking Agent directly to the particle reaction tube.
3. Vortex-mix and ultrasonicate until fully dispersed homogenously.
4. Incubate for 1 hour at room temperature under continuous gentle agitation.

Note: *Particle aggregation may occur. This may be reversed during Step 5: Storage of Conjugated Particles.*

Depending on the characteristics of the particles, the optimal incubation period may vary, and user-driven optimisations may be necessary.

Step 5: Storage of Conjugated Particles

1. Separate the activated particles and remove at least 95% (522 µL) of the supernatant.
2. Add 10 X the initial volume of particle in Step 1.2 (500 µL) of *Storage Buffer* to the particles, vortex mix and ultrasonicate until dispersed homogenously.
3. Repeat steps 1 and 2 twice.
4. Separate the conjugated particles and remove at least 95% (500 µL) of the supernatant.
5. Reconstitute the pellet to the final volume in Step 1.3 (500 µL) by adding *Storage Buffer* to the particles, vortex mix and ultrasonicate until dispersed homogenously.
6. The particles are approximately 10 mg/mL and are ready for use or may be stored at 2 to 8 °C until required.

Note: *The end user must determine the stability of each conjugate in Storage Buffer and whether additives may be required to stabilise the conjugated biomolecule.*

AnteoTech recommends using techniques such as dry weight measurements or absorbance scans to determine the overall particle concentration.

Thoroughly resuspend particles via vortex-mixing and sonication before use.

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.
	Conjugation efficacy suboptimal	Attempt co-conjugation: Add Blocking Agent during *Step 3: Particle Conjugation.* Contact AnteoTech Technical Support for further details.
Non-Specific Signal	Inappropriate blocking	Optimise Blocker Agent. AnteoTech recommends 10% (w/v) BSA in *Conjugation Buffer*. Alternative blocking agents include but are not limited to, casein, fish skin gelatine, and synthetic blockers. Contact AnteoTech Technical Support for further details.
Conjugate not stable	Biomolecule has limited shelf life	AnteoBind™ activation does not protect against biomolecule degradation related to shelf-life limitations. An alternative biomolecule supplier may be required.
	Inappropriate *Conjugation Buffer* used	Use the *Conjugation Buffer* formulation provided. Contact AnteoTech Technical Support for further details.
	Inappropriate *Storage Buffer* used	Use the *Storage Buffer* formulation provided. Additives such as surfactant, other biomolecules and/or sugar may be required for further stability. Contact AnteoTech Technical Support for further details.
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. The *Storage Buffer* may need to be optimised. Contact AnteoTech Technical Support for further details.