AnteoTech

AnteoBind™ - Nano Kit

(For particles < 0.5 µm)

AnteoTech Ltd
Unit 4, 26 Brandl Street,
Eight Mile Plains, Queensland, 4113
Australia

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 Instructions 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. Kit Safety Data Sheet (SDS) is available by contacting AnteoTech Technical Support.
4. Buffers contain ProClin™ 300 or sodium azide as preservatives.
5. Wear appropriate personal protective equipment when using this kit.
6. Follow institutional safety procedures for working with chemicals and handling biological samples.
7. Handle waste as per institutional procedures and in accordance with local regulations.
8. The use of low-binding tubing and micropipette tips is highly recommended to minimise particle loss.
9. Always protect photosensitive particles from light.
10. 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™ is a molecular glue comprised of polymeric metal ions that facilitates conjugation via the utilisation of co-ordination avidity binding between synthetic surfaces and biomolecules. The result is a simplified activation and conjugation process that provides native and secure biomolecule binding.

The AnteoBind™ Nano Kit contains enough reagent to activate and conjugate a total of 10 mg of activate carboxylated particles of < 0.5 µm in diameter. This kit can be used to perform conjugations at scales ranging from 0.2 to 10 mg of particles per reaction. This kit includes the AnteoBind™ conjugation reagents necessary for the successfully conjugation of biomolecules to your particle of interest, although further optimisation is recommended to achieve the best possible result. 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, turbidimetric assays or for lateral flow immunoassay development purposes.

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

Principles of AnteoBind™

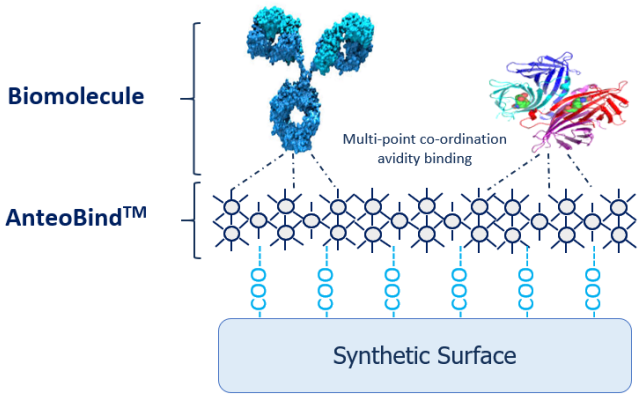


Image left: Schematic representation of AnteoBind™ functioning as a molecular glue, facilitating the conjugation of synthetic surfaces and biomolecules.

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 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.

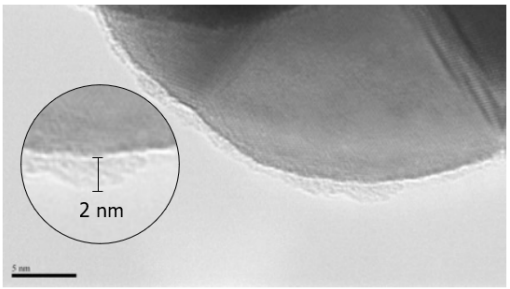


Image left: Transmission electron microscope image demonstrating surface activation. This image of an activated gold nanoparticle demonstrates that the surface is coated in approximately 2 nm of AnteoBind™ and is ready for biomolecule conjugation.

AnteoBind™ activated particles have been demonstrated to remain stable at 2-8 °C for 1 year. With this kit, 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™ kit allows for extended usage and eliminates the need for immediate utilisation.

Provided Materials

Component	Reference	Step	Amount provided
Particle Pre-treatment Solution	A-CMPPPS1	**Optional Step**	1 x 2 mL
Particle Activation Solution	A-CMPPASF1	**Step 1**	1 x 5 mL
Particle Wash Solution	A-CMPWSF1	**Step 2**	1 x 15 mL
Conjugation Buffer (pH 6.0)	A-CMPCBF1	**Step 2 & 3**	2 x 15 mL
Blocker Diluent (pH 6.0)	A-CMPBDF1	**Step 4**	1 x 15 mL
Storage Buffer (pH 8.0)	A-CMPSBF1	**Step 5**	1 x 15 mL

Required Materials – not provided

- Carboxylated particles < 0.5 µm in diameter
- Low binding polypropylene reaction tubes **- Highly Recommended**
- Low binding micropipette tips **- Highly Recommended**
- Biomolecule prepared in *A-CMPCBF1: Conjugation Buffer*
- Preferred Blocking Agent prepared in *A-CMPBDF1: Blocker Diluent*

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

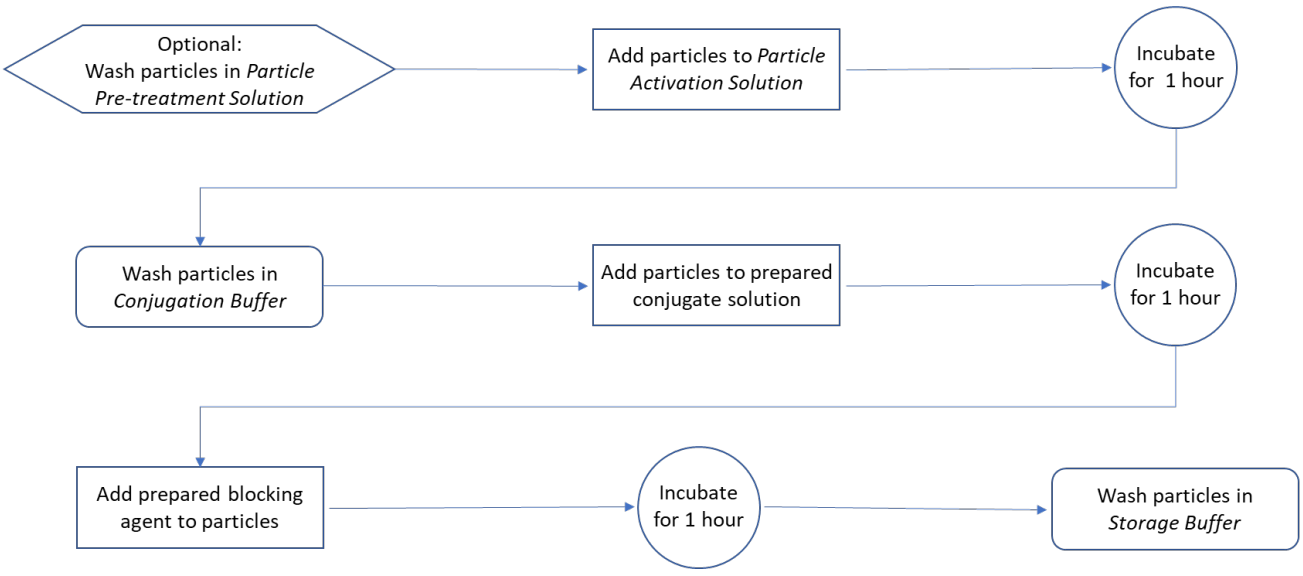
Special Operating Instructions

Biomolecule Compatibility	This kit 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. Particles are recommended to be activated and conjugated at 2 mg/mL. Particles prone to aggregation may be treated with *A-CMPPPS1: Particle Pre-treatment Solution* prior to activation. *A-CMPPPS1: Particle Pre-treatment Solution* contains a large molecular weight, non-ionic surfactant that promotes steric repulsion, preventing particle surface interaction and inhibiting aggregation.

	This kit has been used to conjugate biomolecules to Merck Estapor M1 020/50 and F1-Eu-030 (Note: requires *Optional step: Particle Pre-Treatment*), Allrun PM3-020, Thermo Fisher Scientific Fluoro-Max and Opti-Link particles, amongst other particles.
Biomolecule Concentration	Protein: this kit has been used at ranges of 25 to 200 µg of proteins per mg of particles. Oligonucleotide: this kit has 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	Co-conjugation of two or more biomolecules is possible using AnteoBind™, which 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 molecule/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 scale 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 visually or through the aid of a Dynamic Light Scattering instrument (or equivalent) prior to *Optional step: Particle Pre-Treatment* or *Step 1: Particle Activation* and 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 kit contains *A-CMPBDF1: Blocker Diluent* for Blocking Agent preparation. AnteoTech recommends the use of ≥ 98% pure, protease free bovine serum albumin (BSA) (Merck Product Number: A7030) at 10% (w/v) in *A-CMPBDF1: Blocker Diluent*.

	The optimal Blocking Agent may vary between different particles, conjugates, and assay systems and must be optimised by the user.
Scale	This kit has been successfully used to activate and conjugate particle batches ranging from 0.2 to 10 mg
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 A-*CMPSBF1: 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** *Optional step: Particle Pre-Treatment* or *Step 1: Particle Activation* **and after** *Step 2: Activated Particle Wash* and *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 with this kit and to determine the effectiveness of the activation and conjugation processes. ***Note:*** *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

The following procedure details the general process for the activation of 1 mg of carboxylated particles < 0.5 µm in diameter with AnteoBind™ followed by biomolecule conjugation.

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 adjusted to 20 mg/mL prior to activation, if not, 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 with *Particle Wash Solution* prior to *Step 1: Particle Activation*. 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 are determined prior to *Optional step: Particle Pre-Treatment* or *Step 1: Particle Activation* and after *Step 2: Activated Particle Wash* and *Step 5: Storage of Conjugated Particles*.

Optional step: Particle Pre-Treatment

Note: *Pre-treatment is only recommended for unstable particles that are prone to aggregation. Particles that are unable to maintain the stability of a colloidal system are generally stored in surfactants, such as but not limited to, Tween-20.*

1. Transfer 50 µL (1 mg) of particles to a new reaction tube.
2. Separate the particles until the supernatant becomes clear and remove the supernatant.
3. Transfer 50 µL of *Particle Pre-treatment Solution* to the reaction tube.
4. 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.*

Step 1: Particle Activation

1. Transfer 450 µL of *Particle Activation Solution* to a new reaction tube.
2. Transfer 50 µL (1 mg) of particles (or particles from the optional *Particle Pre-treatment* step) 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.
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.*

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

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 *Particle Wash Solution* to the particles, vortex mix and ultrasonicate until dispersed homogenously.
3. Separate the activated particles from solution and remove at least 95% (475 µL) of the supernatant.
4. 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.
5. Repeat steps 2.3 and 2.4.
6. Separate the activated particles from solution as per particle manufacturer's instructions and remove at least 95% (475 µL) of the supernatant.
7. Reconstitute the pellet to half the final volume in step 2.2 (250 µL) by adding *Conjugation Buffer* to the particles, vortex mix and ultrasonicate until dispersed homogenously.

Step 3: Particle Conjugation

1. Prepare the biomolecule to be conjugated at the required concentration in the same final volume as in Step 2.5 (250 µL) of *Conjugation Buffer*.

Example 1: *For conjugation at 25 µg of biomolecule per mg particles, prepare 250 µL of 100 µg/mL biomolecule.*

Example 2: *For conjugation at 200 µg of biomolecule per mg particles, prepare 250 µL of 800 µg/mL biomolecule.*

Target biomolecule to be conjugated per mg of particles (µg/mg)	**Biomolecule concentration prepared in 250 µL of *Conjugation Buffer* (µg/mL)**
25	100
50	200
100	400
200	800

Note: *As a general rule, if the target biomolecule loading per mg of particles is **X** µg, the biomolecule should be prepared at 4**X** µg/mL in Conjugation Buffer.*

2. Add the entirety of the contents (> 250 µL) from the washed particle tube (Step 2.5) to the prepared conjugate (Step 3.1). Vortex-mix until fully dispersed homogenously.
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.*

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 conjugation process.*

Step 4: Particle Blocking

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

Note: *AnteoTech recommends using Merck Product Number: A7030 BSA at10% (w/v) in Blocker Diluent 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 Blocking Agent at the required concentration in 100 µL of *Blocker Diluent*.
2. Add 10% of the total volume in Step 1.3 (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.*

Note: *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% (523 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 5.1 and 5.2.
4. Separate the conjugated particles and remove at least 95% (475 µL) of the supernatant.
5. Reconstitute the pellet to 20% of the final volume in step 1.3 (100 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 additive may be required to stabilise the conjugated biomolecule.*

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

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

Magnetic particle example procedure 1

The following procedure details AnteoTech's optimised process for the activation of 1 mg of unstable 200 nm superparamagnetic polystyrene particles with AnteoBind™, followed by Cardiac Troponin I monoclonal antibody (cTnI mAB) conjugation.

Particle Pre-Treatment

1. Determine raw material particle diameter, polydispersity index via Dynamic Light Scattering analysis and zetapotential via Laser Doppler Electrophoresis.
2. Transfer 50 µL (1 mg) of 20 mg/mL particles to a 1.7 mL reaction tube.
3. Separate the particles on a magnetic tube rack for 1 minute.
4. Remove and discard the supernatant taking care not to disturb the pellet.
5. Transfer 50 µL of *Particle Pre-treatment Solution* to the reaction tube.
6. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the sweet spot of a water bath.

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.*

Step 1: Particle Activation

1. Transfer 450 µL of *Particle Activation Solution* to a new reaction tube.
2. Spot centrifuge the pre-treatment reaction tube and then transfer the particles to the *Particle Activation Solution* reaction tube.
3. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
4. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 2: Activated particle wash

1. Spot centrifuge the reaction tube and then separate the particles on a magnetic tube rack for 1 minute.
2. Remove and discard the supernatant taking care not to disturb the pellet.
3. Add 500 µL of *Particle Wash Solution* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
4. Spot centrifuge the reaction tube and then separate the particles on a magnetic tube rack for 1 minute.
5. Remove and discard the supernatant taking care not to disturb the pellet.
6. Add 500 µL of *Conjugation Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
7. Repeat steps 4 to 6.
8. Spot centrifuge the reaction tube and then separate the particles on a magnetic tube rack for 1 minute.
9. Remove and discard the supernatant taking care not to disturb the pellet.
10. Add 250 µL of *Conjugation Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
11. Determine activated particle diameter, polydispersity index via Dynamic Light Scattering analysis and zetapotential via Laser Doppler Electrophoresis.

Step 3: Particle Conjugation

1. Prepare 200 µg/mL cTnI mAb with 200 µg/mL BSA in 250 µL of *Conjugation Buffer*.
2. Add the 250 µL of washed particles to the 250 µL of conjugation solution.
3. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
4. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 4: Particle Blocking

1. Prepare 10% (w/v) BSA in *Blocker Diluent*.
2. Add 50 µL of the 10% (w/v) BSA directly to the particle reaction tube.
3. Vortex-mix for 10 seconds and then water bath ultrasonicate for 5 minutes in the water bath sweet spot.
4. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 5: Storage of Conjugated Particles

1. Spot centrifuge the reaction tube and then separate the particles on a magnetic tube rack for 1 minute.
2. Remove and discard the supernatant taking care not to disturb the pellet.
3. Add 500 µL of *Storage Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
4. Repeat steps 1 to 3.
5. Spot centrifuge the reaction tube and then separate the particles on a magnetic tube rack for 1 minute.
6. Remove and discard the supernatant taking care not to disturb the pellet.
7. Add 100 µL of *Storage Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.

8. Determine conjugated particle diameter, polydispersity index via Dynamic Light Scattering analysis and zetapotential via Laser Doppler Electrophoresis.
9. The particles are approximately 10 mg/mL and are ready for use.

Magnetic particle example procedure 2

The following procedure details AnteoTech's optimised process for the activation of 1 mg of unstable 300 nm superparamagnetic polystyrene particles with AnteoBind™, followed by Poly dT 30-mer oligonucleotide conjugation.

Particle Pre-Treatment

1. Determine raw material particle diameter, polydispersity index via Dynamic Light Scattering analysis and zetapotential via Laser Doppler Electrophoresis.
2. Transfer 50 µL (1 mg) of 20 mg/mL particles to a 1.7 mL reaction tube.
3. Separate the particles on a magnetic tube rack for 1 minute.
4. Remove and discard the supernatant taking care not to disturb the pellet.
5. Transfer 50 µL of *Particle Pre-treatment Solution* to the reaction tube.
6. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the sweet spot of a water bath.

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.*

Step 1: Particle Activation

1. Transfer 450 µL of *Particle Activation Solution* to a new reaction tube.
2. Spot centrifuge the pre-treatment reaction tube and then transfer the particles to the *Particle Activation Solution* reaction tube.
3. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
4. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 2: Activated particle wash

1. Spot centrifuge the reaction tube and then separate the particles on a magnetic tube rack for 1 minute.
2. Remove and discard the supernatant taking care not to disturb the pellet.
3. Add 500 µL of *Particle Wash Solution* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
4. Spot centrifuge the reaction tube and then separate the particles on a magnetic tube rack for 1 minute.
5. Remove and discard the supernatant taking care not to disturb the pellet.
6. Add 500 µL of *Conjugation Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
7. Repeat steps 4 to 6.
8. Spot centrifuge the reaction tube and then separate the particles on a magnetic tube rack for 1 minute.
9. Remove and discard the supernatant taking care not to disturb the pellet.
10. Add 250 µL of *Conjugation Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
11. Determine activated particle diameter, polydispersity index via Dynamic Light Scattering analysis and zetapotential via Laser Doppler Electrophoresis.

Step 3: Particle Conjugation

1. Prepare 16,000 pmol/mL Poly dT 30-mer oligonucleotide in 250 µL of *Conjugation Buffer*.
2. Add the 250 µL of washed particles to the 250 µL of conjugation solution.
3. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
4. Incubate for 2 hours at room temperature on a tube rotator at 25 rpm.

Step 4: Particle Blocking

1. Prepare 10% (w/v) BSA in *Blocker Diluent*.
2. Add 50 µL of the 10% (w/v) BSA directly to the particle reaction tube.
3. Vortex-mix for 10 seconds and then water bath ultrasonicate for 5 minutes in the water bath sweet spot.
4. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 5: Storage of Conjugated Particles

1. Spot centrifuge the reaction tube and then separate the particles on a magnetic tube rack for 1 minute.
2. Remove and discard the supernatant taking care not to disturb the pellet.
3. Add 500 µL of *Storage Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
4. Repeat steps 1 to 3.
5. Spot centrifuge the reaction tube and then separate the particles on a magnetic tube rack for 1 minute.
6. Remove and discard the supernatant taking care not to disturb the pellet.
7. Add 100 µL of *Storage Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 5 minutes in the water bath sweet spot.
8. Determine conjugated particle diameter, polydispersity index via Dynamic Light Scattering analysis and zetapotential via Laser Doppler Electrophoresis.
9. The particles are approximately 10 mg/mL and are ready for use.

Non-magnetic particle example procedure

The following procedure details AnteoTech's optimised process for the activation of 1 mg of 300 nm black dyed polystyrene particles with AnteoBind™, followed by Cardiac Troponin I monoclonal antibody (cTnI mAB) conjugation.

Step 1: Particle Activation

1. Determine raw material particle diameter, polydispersity index via Dynamic Light Scattering analysis and zetapotential via Laser Doppler Electrophoresis.
2. Transfer 450 µL of *Particle Activation Solution* to a 1.7 mL reaction tube.
3. Transfer 50 µL (1 mg) of 20 mg/mL particles to the reaction tube.
4. Vortex-mix for 10 seconds and then ultrasonicate for 3 rounds of 10 seconds in a solid medium ultrasonicator.
5. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 2: Activated particle wash

1. Separate the particles via centrifugation at 10,000 RCF, 20 °C and for 10 minutes.
2. Remove 475 µL of the supernatant and discard taking care not to disturb the pellet.
3. Add 475 µL of *Particle Wash Solution* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 3 rounds of 10 seconds in the titanium sonotrode.

4. Separate the particles via centrifugation at 10,000 RCF, 20 °C and for 10 minutes.
5. Remove 475 µL of the supernatant and discard taking care not to disturb the pellet.
6. Add 475 µL of *Conjugation Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 3 rounds of 10 seconds in the titanium sonotrode.
7. Repeat steps 4 to 6.
8. Separate the particles via centrifugation at 10,000 RCF, 20 °C and for 10 minutes.
9. Remove 475 µL of the supernatant and discard taking care not to disturb the pellet.
10. Add 225 µL of *Conjugation Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 3 rounds of 10 seconds in the titanium sonotrode.
11. Determine activated particle diameter, polydispersity index via Dynamic Light Scattering analysis and zetapotential via Laser Doppler Electrophoresis.

Step 3: Particle Conjugation

1. Prepare 400 µg/mL cTnI mAb with 200 µg/mL BSA in 250 µL of *Conjugation Buffer*.
2. Add the 250 µL of washed particles to the 250 µL of conjugation solution.
3. Vortex-mix for 10 seconds and then ultrasonicate for 3 rounds of 10 seconds in the titanium sonotrode.
4. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 4: Particle Blocking

1. Prepare 10% (w/v) BSA in *Blocker Diluent*.
2. Add 50 µL of the 10% (w/v) BSA directly to the particle reaction tube.
3. Vortex-mix for 10 seconds and then ultrasonicate for 3 rounds of 10 seconds in the titanium sonotrode.
4. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 5: Storage of Conjugated Particles

1. Separate the particles via centrifugation at 10,000 RCF, 20 °C and for 10 minutes.
2. Remove 475 µL of the supernatant and discard taking care not to disturb the pellet.
3. Add 475 µL of *Storage Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 3 rounds of 10 seconds in the titanium sonotrode.
4. Repeat steps 1 to 3.
5. Separate the particles via centrifugation at 10,000 RCF, 20 °C and for 10 minutes.
6. Remove 475 µL of the supernatant and discard taking care not to disturb the pellet.
7. Add 75 µL of *Storage Buffer* to the particles. Vortex-mix for 10 seconds and then ultrasonicate for 3 rounds of 10 seconds in the titanium sonotrode.
8. Determine conjugated particle diameter, polydispersity index via Dynamic Light Scattering analysis and zetapotential via Laser Doppler Electrophoresis.
9. The particles are approximately 10 mg/mL and are ready for use.

Troubleshooting

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	Complete *Optional Step: Particle Pre-Treatment* prior to particle activation. Contact AnteoTech Technical Support is issue persists.
	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 Blocking Agent. AnteoTech recommends 10% (w/v) BSA in the provided *A-CMPBDF1: Blocker Diluent*. 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 provided with the kit. Contact AnteoTech Technical Support for further details.
	Inappropriate Storage Buffer used	Use the Storage Buffer provided with the kit. 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.

© 2023 AnteoTech Group of Companies. All Rights Reserved.
AnteoBind™ is a trademark of the AnteoTech Group of Companies.