AnteoTech

Activation Kit Multiplex Microspheres

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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.

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. Kit Safety Data Sheet (SDS) is available by contacting AnteoTech Technical Support.
4. Kit components contain ProClin™ 300 as a preservative.
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. 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.

Activation Kit Multiplex Microspheres Publications

To access publications that have utilised the Activation Kit Multiplex Microsphere kit please visit the Activation Kit Luminex Beads section of our Publications webpage https://www.anteotech.com/life-science/anteobind/publications/.

Description

The Activation Kit Multiplex Microspheres contains the reagents necessary to activate Luminex® MagPlex® or MicroPlex® Microspheres with AnteoBind™ and to then conjugate biomolecule(s) to the activated microspheres. 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 native and secure biomolecule binding.

This kit is available in 10, 40 and 400 reaction configurations. A standard reaction is defined as the activation and conjugation of 1.25 million microspheres; however, this kit can be used at scales ranging from 0.625 million to 12.5 million microspheres per reaction. The conjugated microspheres can then be used with Luminex® xMAP® technology to develop monoplex or multiplex assays.

This kit contains an optimised buffer set that allows the user to focus on the vital elements of conjugation optimisation. By removing the requirement of buffer optimisation, the kit allows users developing novel assays to perform numerous small-scale conjugations to test multiple biomolecule and blocking parameters.

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™

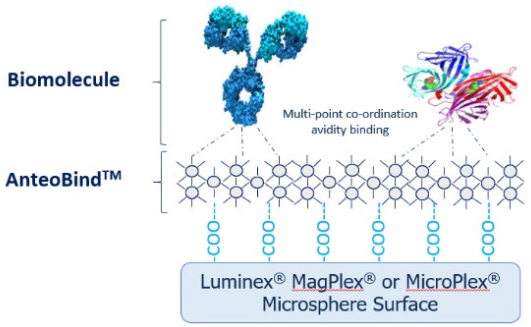


Image left: Schematic representation of AnteoBind functioning as a molecular glue, facilitating the conjugation of synthetic surfaces and biomolecules.

The conjugation process involves two major steps, microsphere 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 microsphere surface, in essence 'activating' the microsphere surface, priming it for native and secure biomolecule binding.

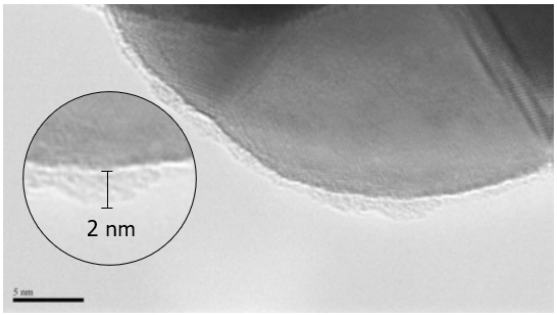


Image left: Transmission electron microscope image demonstrating microsphere activation. The microsphere is coated in approximately 2 nm of AnteoBind and is ready for biomolecule conjugation.

AnteoBind activated microspheres have been demonstrated to remain stable for 1 year. This kit allows the user to either activate microspheres in bulk or multiple small lots using the same reagents, providing the user with enhanced experimental reproducibility. This differs from conventional covalent conjugation chemistry where the components must be used immediately after reconstitution.

Provided Materials

Component	Reference	Step	Amount provided		
			10 reactions	40 reactions	400 reactions
AnteoBind™ Activation Reagent	A-CMPARA1	**Step 1**	1 x 1 mL	4 x 1 mL	1 x 40 mL
Conjugation Buffer (pH 5.2)	A-CMPCBB1	**Step 2 & 3**	1 x 15 mL	2 x 15 mL	1 x 200 mL
Storage Buffer	A-CMPSBB1	**Step 4**	1 x 15 mL	2 x 15 mL	1 x 200 mL

Required Materials – not provided

- Luminex® MagPlex® or MicroPlex® Microspheres
- Low binding polypropylene reaction tubes
- Low binding micropipette tips
- Biomolecule prepared in *A-CMPCBB1: Conjugation Buffer*
- Optional: Blocker Agent prepared in *A-CMPCBB1: Conjugation Buffer*

Suggested Equipment

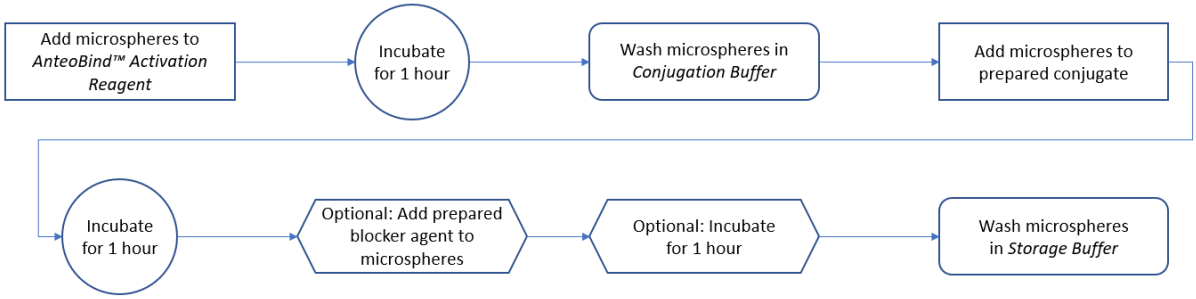
Process	Equipment required
Microsphere dispersion	Ultrasonicator (liquid or solid medium)
Separation of MicroPlex® Microspheres	Centrifuge
Separation of MagPlex® Microspheres	Magnetic tube rack
Solution and buffer transfer/supernatant removal	Micropipettes
Microsphere incubation	Tube rotator, roller, or mixer
Sample mixing	Vortex mixer
Sample spot centrifugation	Microcentrifuge

Special Operating Instructions

Biomolecule compatibility	Biomolecule compatibility is not guaranteed and must be determined by the user. Co-conjugation of multiple biomolecules is possible. AnteoTech recommends the addition of equal amounts of biomolecule during *Step 2: Microsphere Conjugation*.
Microsphere compatibility	This kit is compatible with Luminex® MagPlex® and MicroPlex® Microspheres.
Conjugate concentration	This kit is compatible with protein conjugation at 2 to 20 µg per million microspheres. AnteoTech recommends 2 µg per million microspheres as a conjugation starting point. Optimal conjugate concentration is dependent on biomolecule type and must be determined by the user.
Microsphere separation	MagPlex® Microspheres: magnetic separator required. Luminex® recommends a 1 to 2-minute magnetic separation timeframe.

	Separation parameters should be optimised by the user. MicroPlex® Microspheres: centrifugation required. Luminex® recommends centrifugation at ≥ 8,000 x g for 1 to 2 minutes. Centrifugation parameters should be optimised by the user.
Microsphere aggregation	Pipette-mix, vortex-mix and/or ultrasonicate microspheres until dispersed. Ultrasonication parameters must be optimised by the user.
Optional blocker preparation	This kit contains *A-CMPCBB1: Conjugation Buffer* for optional blocker agent preparation. In general, AnteoTech recommends the use of ≥ 98% pure, protease free bovine serum albumin (BSA) at 0.1% (w/v) in *A-CMPCBB1: Conjugation Buffer*. For biomolecules < 65 kDa AnteoTech recommends the use of 2 to 5 kDa synthetic blocker agents (e.g. Blockmaster™ CE510/CE210). The optimal blocker agent may vary between different conjugate types and must be optimised by the user.
Scale	This kit has been tested at scales ranging from 0.625 million to 12.5 million microspheres per reaction.
Conjugated microsphere storage and stability	If not immediately used AnteoTech recommends storage of conjugated microspheres at 2-8 °C under continuous gentle agitation (e.g. tube rotator, mixer, or roller at 25 rpm). The user must determine the stability of each conjugate in *A-CMPSBB1: Storage Buffer*.

Process Workflow



General Procedure

The following procedure details the general process for the activation of 1.25 million Luminex® MagPlex® or MicroPlex® Microspheres with AnteoBind followed by protein conjugation.

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

Before commencing please note:

- **Microsphere stock must be 12.5 million microspheres per mL.**
- Ensure all materials are at room temperature before use.
- Use a micropipette to remove supernatant taking care not to disturb the microsphere pellet.
- Vortex-mix microspheres prior to use. Ultrasonicate if required.

Step 1: Microsphere Activation

1. Transfer 100 µL (1.25 million) of microspheres to a 1.7 mL reaction tube.
2. Separate the microspheres and remove the supernatant.
3. Add 100 µL of *A-CMPARA1: AnteoBind™ Activation Reagent* to the reaction tube.
4. Vortex-mix and ultrasonicate until dispersed.
5. Incubate for 1 hour at room temperature under continuous gentle agitation (e.g. tube rotator at 25 rpm).

Step 2: Microsphere Conjugation

1. Separate the microspheres and remove the supernatant.
2. Add 100 µL of *A-CMPCBB1: Conjugation Buffer* to the microspheres, vortex mix and ultrasonicate until dispersed.
3. Repeat steps 1 and 2.
4. Prepare biomolecule at the required concentration in 100 µL of *A-CMPCBB1: Conjugation Buffer*.

Note: *AnteoTech recommends 2 µg per million microspheres (100 µL of 25 µg/mL) as a starting point.*

5. Separate the microspheres and remove the supernatant.
6. Add the prepared conjugate, vortex-mix and ultrasonicate until dispersed.
7. Incubate for 1 hour at room temperature under continuous gentle agitation (e.g. tube rotator at 25 rpm).

Step 3 (Optional): Microsphere Blocking

Note: *The optimal blocker agent may vary between different microspheres and conjugate types. Please refer to Special Operating Instructions for guidance.*

1. Separate the microspheres and remove the supernatant.
2. Add 100 µL of *A-CMPCBB1: Conjugation Buffer* to the microspheres, vortex mix and ultrasonicate until dispersed.
3. Repeat steps 1 and 2.
4. Prepare blocker agent at the required concentration in 100 µL of *A-CMPCBB1: Conjugation Buffer*.
5. Separate the microspheres and remove the supernatant.
6. Add the prepared blocker agent, vortex-mix and ultrasonicate until dispersed.
7. Incubate for 1 hour at room temperature under continuous gentle agitation (e.g. tube rotator at 25 rpm).

Step 4: Storage of Conjugated Microspheres

1. Separate the microspheres and remove the supernatant.
2. Add 100 µL of *A-CMPSBB1: Storage Buffer* to the microspheres, vortex mix and ultrasonicate until dispersed.
3. Repeat steps 1 and 2 twice.

Note: *The end user must determine the stability of each conjugate in A-CMPSBB1: Storage Buffer.*

Step 5 (Optional): Conjugation Assessment

1. Luminex® strongly recommends the enumeration of microspheres via a cell counter or hemocytometer. Please refer to the cell counter or hemocytometer's user manual for instructions.
2. Luminex® strongly recommends the assessment of conjugation efficiency prior to proceeding to assay development. Please refer to the 'Confirm Sample Coupling Protocol' section of the Luminex® xMAP® Antibody Coupling Kit Package Insert for instructions.

MagPlex® Microsphere example procedure #1

The following procedure details AnteoTech's optimised process for the activation of 1.25 million MagPlex® Microspheres with AnteoBind followed by conjugation with SARS-CoV-2 Nucleocapsid Protein antigen.

Step 1: Microsphere Activation

1. Transfer 100 µL (1.25 million) of microspheres to a 1.7 mL reaction tube.
2. Spot centrifuge the reaction tube and then separate the microspheres on a magnetic tube rack for 1 minute.
3. Remove and discard the supernatant taking care not to disturb the pellet.
4. Add 100 µL of *A-CMPARA1: AnteoBind™ Activation Reagent* to the reaction tube.
5. Vortex-mix for 10 seconds and then ultrasonicate for 1 minute 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.*

6. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 2: Microsphere Conjugation

1. Spot centrifuge the reaction tube and then separate the microspheres on a magnetic tube rack for 1 minute.
2. Remove and discard the supernatant taking care not to disturb the pellet.
3. Add 100 µL of *A-CMPCBB1: Conjugation Buffer* to the microspheres. Vortex-mix for 10 seconds and then ultrasonicate for 1 minute in the water bath sweet spot.
4. Repeat steps 1 to 3.
5. Prepare 62.5 µg/mL in 100 µL of *A-CMPCBB1: Conjugation Buffer*.
6. Spot centrifuge the reaction tube and then separate the microspheres on a magnetic tube rack for 1 minute.
7. Remove and discard the supernatant taking care not to disturb the pellet.
8. Add the prepared conjugate to the reaction tube. Vortex-mix for 10 seconds and then ultrasonicate for 1 minute in the water bath sweet spot.
9. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 3: Storage of Conjugated Microspheres

1. Spot centrifuge the reaction tube and then separate the microspheres on a magnetic tube rack for 1 minute.
2. Add 100 µL of *A-CMPSBB1: Storage Buffer* to the microspheres. Vortex-mix for 10 seconds and then ultrasonicate for 1 minute in the water bath sweet spot.
3. Repeat steps 1 and 2 twice.

MagPlex® Microsphere example procedure #2

The following procedure details AnteoTech's optimised process for the activation of 0.625 million MagPlex® Microspheres with AnteoBind followed by co-conjugation with monoclonal Mouse Anti-Human Tumour Necrosis Factor (TNF) and monoclonal Rat Anti-Human Interleukin 6 (IL-6).

Step 1: Microsphere Activation

1. Transfer 50 µL (0.625 million) of microspheres to a 1.7 mL reaction tube.
2. Spot centrifuge the reaction tube and then separate the microspheres on a magnetic tube rack for 1 minute.
3. Remove and discard the supernatant taking care not to disturb the pellet.
4. Add 50 µL of *A-CMPARA1: AnteoBind™ Activation Reagent* to the reaction tube.
5. Vortex-mix for 10 seconds and then ultrasonicate for 1 minute 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.*

6. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 2: Microsphere Conjugation

1. Spot centrifuge the reaction tube and then separate the microspheres on a magnetic tube rack for 1 minute.
2. Remove and discard the supernatant taking care not to disturb the pellet.
3. Add 50 µL of *A-CMPCBB1: Conjugation Buffer* to the microspheres. Vortex-mix for 10 seconds and then ultrasonicate for 1 minute in the water bath sweet spot.
4. Repeat steps 1 to 3.
5. Prepare 25 µg/mL Mouse TNF and 25 µg/mL Rat IL-6 in 50 µL of *A-CMPCBB1: Conjugation Buffer*.
6. Spot centrifuge the reaction tube and then separate the microspheres on a magnetic tube rack for 1 minute.
7. Remove and discard the supernatant taking care not to disturb the pellet.
8. Add the prepared conjugate to the reaction tube. Vortex-mix for 10 seconds and then ultrasonicate for 1 minute in the water bath sweet spot.
9. Incubate for 1 hour at room temperature on a tube rotator at 25 rpm.

Step 3: Storage of Conjugated Microspheres

1. Spot centrifuge the reaction tube and then separate the microspheres on a magnetic tube rack for 1 minute.
2. Add 50 µL of *A-CMPSBB1: Storage Buffer* to the microspheres. Vortex-mix for 10 seconds and then ultrasonicate for 1 minute in the water bath sweet spot.
3. Repeat steps 1 and 2 twice.

Trouble Shooting

Issue	Possible Cause(s)	Recommendations
Mild Aggregation	Insufficient sample mixing	Vortex mix samples for at least 10 seconds before use.
	Microspheres not appropriately dispersed	Optimise ultrasonication parameters. AnteoTech recommends liquid medium ultrasonication at 384W or solid medium ultrasonication at 12W.
Non-specific signal	Inappropriate blocker agent	In general, AnteoTech recommends 0.1% w/v BSA in *A-CMPCBB1: Conjugation Buffer*. AnteoTech recommends 2 to 5 kDa synthetic blocker agents for biomolecules < 65 kDa. Contact AnteoTech Technical Support for further details.
Poor conjugation efficiency	Inappropriate Conjugation Buffer used	Use the Conjugation Buffer provided with the kit. The addition of detergents (e.g. Tween-20), metal chelators (e.g. EDTA) or high phosphate concentrations should be avoided. If biomolecules are not stable at pH 5.2, alternative buffer systems (e.g. HEPES) may be required. 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. The addition of detergents, metal chelators or phosphates should be avoided. If biomolecules are not stable at pH 5.2, alternative buffer systems (e.g. HEPES) may be required. Contact AnteoTech Technical Support for further details.
	Inappropriate Storage Buffer used	Use the Storage Buffer provided with the kit. The addition of phosphates should be avoided. Alternative buffer systems (e.g. HEPES, TRIS) may be required. Contact AnteoTech Technical Support for further details.
Loss of microspheres	Inappropriate centrifugal separation	Use appropriate centrifuge settings and rotors for the sample volumes being processed. Contact AnteoTech Technical Support for further details.
	Inappropriate magnetic separation	Ensure that the magnetic separator is appropriate for the sample volume being processed. Limit the exposure of microspheres to magnetic forces. Overexposure may induce permanent magnetisation and irreversible aggregation of microspheres. Contact AnteoTech Technical Support for further details.
	Pipetting	Take care not to disturb the microsphere pellet during supernatant removal. Do not directly aim the pipette tip at the pellet or excessively agitate the supernatant.
	Prolonged storage	Resuspend microspheres 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.