



AnteoBind™ Biosensor











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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. Reagent 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. 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.

AnteoBind™ Biosensor Publications

A selection of publications on various planar surfaces is outlined below:

Planar Surface	Reference
Cyclic olefin copolymer plastic	<p>Ooi, H. W., Cooper, S. J., Huang, C. Y., Jennins, D., Chung, E., Maeji, N. J., & Whittaker, A. K. (2014). Coordination complexes as molecular glue for immobilization of antibodies on cyclic olefin copolymer surfaces. <i>Analytical biochemistry</i>, 456, 6–13.</p> <p>https://doi.org/10.1016/j.ab.2014.03.023</p>
Glass	<p>Karimian, Tina, Roland Hager, Andreas Karner, Julian Weghuber, and Peter Lanzerstorfer. (2022) A Simplified and Robust Activation Procedure of Glass Surfaces for Printing Proteins and Subcellular Micropatterning Experiments <i>Biosensors</i> 12, no. 3: 140.</p> <p>https://doi.org/10.3390/bios12030140</p>
Polystyrene	<p>Barnett, J. M., Monnier, B. M., Tyler, S., West, D., Ballantine-Dykes, H., Regan, E., Luxton, R. (2020). Initial trail results of a magnetic biosensor for the rapid detection of Porcine Reproductive and Respiratory Virus (PRRSV) infection. <i>Sensing and Bio-Sensing Research</i>, 27, 100315.</p> <p>https://doi.org/10.1016/j.sbsr.2019.100315</p>
Carbon Nanotube	<p>Sánchez-Tirado, E., Salvo, C., González-Cortés, A., Yáñez-Sedeño, P., Langa, F., & Pingarrón, J. M. (2017). Electrochemical immunosensor for simultaneous determination of interleukin-1 beta and tumor necrosis factor alpha in serum and saliva using dual screen-printed electrodes modified with functionalized double-walled carbon nanotubes. <i>Analytica chimica acta</i>, 959, 66–73.</p> <p>https://doi.org/10.1016/j.aca.2016.12.034</p>

Description

AnteoBind™ Biosensor activates planar surfaces 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 activation and conjugation process that provides native and secure biomolecule binding.

This reagent is available in 5 mL, 10 mL, 50 mL, and custom volume configurations. This reagent is recommended for the activation of cyclic olefin copolymer (COC) plastic, glass, polystyrene, carbon nanotubes, silicon oxide, titanium oxide and ceramic planar surfaces that are commonly used in biosensors.

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™

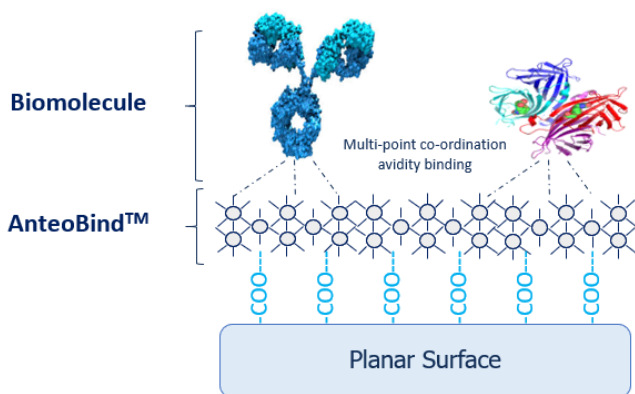


Image left: Schematic representation of AnteoBind™ functioning as a molecular glue, facilitating the conjugation of synthetic surfaces and biomolecules.

The conjugation process involves two major steps, planar surface 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 nanometre thin molecular glue on the planar surface, in essence 'activating' the planar surface, priming it for secure biomolecule binding in native conformations.

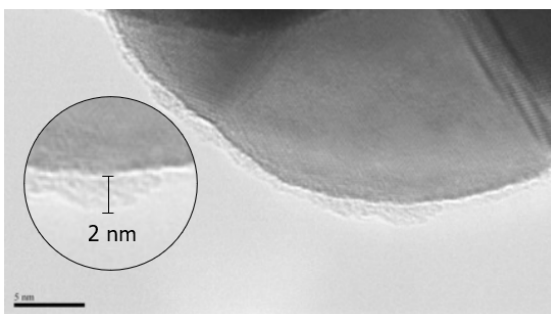


Image left: Transmission electron microscope image demonstrating surface activation. This image of an activated gold nanoparticle demonstrates that the surface is coated in approximately 2 nm of AnteoBind™ and is ready for biomolecule conjugation.



Provided Materials

Component	Reference	Step	Amount provided			
			5 mL	10 mL	50 mL	Custom
AnteoBind™ Biosensor	A-PLSC010	Step 1	1 x 5 mL	1 x 10 mL	1 x 50 mL	Variable

Required Materials – not provided

- Planar surface (e.g. cyclic olefin copolymer (COC) plastic, glass, polystyrene, carbon nanotubes, silicon oxide, titanium oxide and ceramic)
- Low binding fluid transfer equipment/consumables
- Type 1 water (Step 1)
- 25 mM MES pH 6.0 + 0.05% ProClin™ 300 - recommended *Coating Buffer* (Steps 2 & 3)
- 10 mM PBS pH 7.4 with 0.05% Tween20® - recommended *Wash buffer* (Steps 2 & 3)
- Biomolecule prepared in *Coating Buffer* (Step 2)
- Blocking Agent prepared in *Coating Buffer* (Step 3)

Suggested Equipment

Process	Equipment required
Activation, conjugation and blocking	Orbital shaker

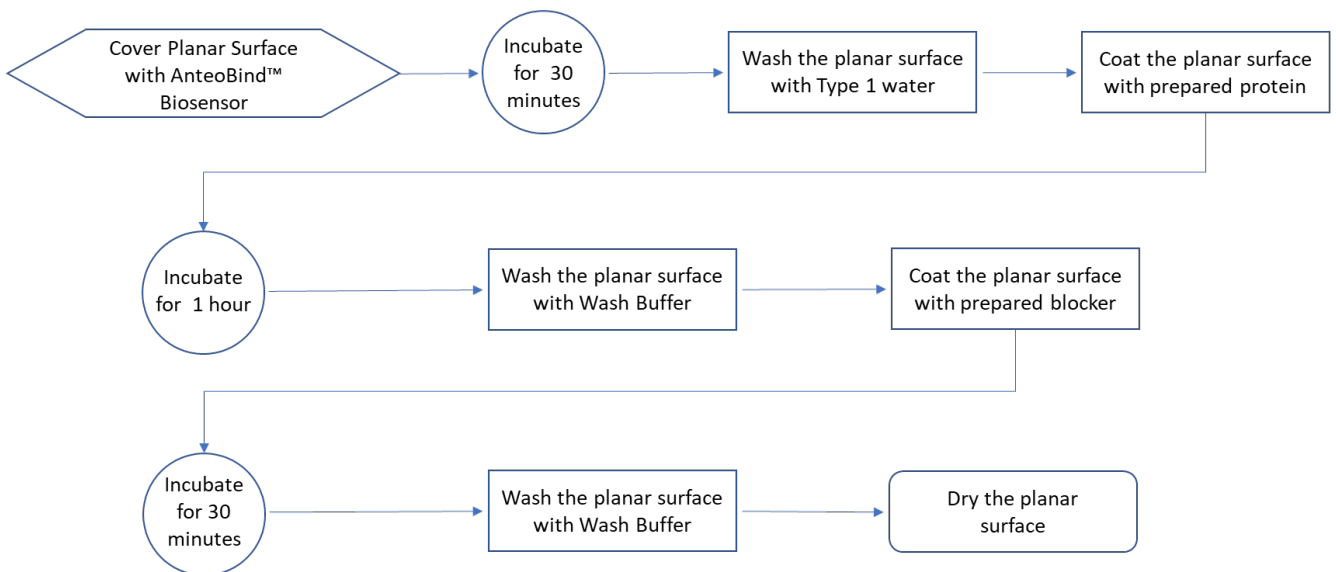
Special Operating Instructions

Biomolecule Compatibility	This reagent has been used to conjugate antibodies, antigens, Fab fragments, streptavidin and Protein A/G. Biomolecule compatibility may vary and must be determined by the user.
Planar Surface Compatibility	AnteoBind™ Biosensor is compatible with cyclic olefin copolymer plastic planar surfaces and is recommended for use with glass, polystyrene, carbon nanotube, silicon oxide, titanium oxide and ceramic planar surfaces. Compatibility is dependent on biomolecule and surface type and must be determined by the user.
Optimal Washing Conditions	Depending on the properties of the target surface, vigorous washing during the recommended wash steps may strip AnteoBind™ from the surface. AnteoTech recommends a gentle washing process to be applied, such as immersion of the surface in Type 1 water or Wash Buffer followed by placement on an orbital shaker set at 25 rpm for 1 minute. For spot coating processes please dispense sufficient liquid to coat the area of interest and gently agitate by orbital shaker or by hand for 1 minute before pipetting or decanting the solution.
Conjugate Concentration	Optimal conjugate concentration is dependent on biomolecule and planar surface type and must be determined by the user.
Incubation Times	Optimal activation, conjugation and blocking incubation times must be determined by the user.



Activated Planar Surface Storage	Activated surfaces should be stored at 2-8 °C under low humidity conditions (with desiccant) if not used immediately.
Buffer Compatibility	While the recommended buffers can be modified with additives depending on the planar surface and/or the biomolecule to be utilised, avoid the use of additives such as detergents (e.g. Tween-20), metal chelators (e.g. EDTA) or high phosphate concentrations, during biomolecule conjugation. <i>Coating Buffer</i> is recommended for Blocking Agent preparation. The optimal blocking solution may be dependent on different planar surfaces, conjugated protein and assay systems and must be optimised by the user.
Blocker Preparation	If uncertain, AnteoTech recommends the use of $\geq 98\%$ pure, protease free bovine serum albumin (BSA) at 5% (w/v) prepared in <i>Coating Buffer</i> (Merck Product Number: A7030) as a starting point when working with antibodies. Smaller molecular weight blockers at lower working concentrations are recommended when working with smaller biomolecules (oligos, antigens etc.)
Drying Process	While air drying under laminar flow is sufficient for most analytes and surfaces end users may opt to dry their surfaces using compressed air or gas with the inclusion of heating. AnteoTech recommends conducting the drying steps according to the practices that best suit the user's application.
Automation	AnteoBind™ Biosensor may be incorporated in automated apparatuses for surface coating of surfaces, however, AnteoTech recommends manual validation and confirmation of procedures before introducing AnteoBind™ Biosensor to automated procedures.

Process Workflow





General Procedure

The following procedure details the general process for the activation of a planar surface with AnteoBind™ Biosensor followed by biomolecule conjugation.

Please familiarise yourself with the **Special Operating Instructions** above prior to the commencement of this procedure.

Before commencing please note:

- Ensure all materials are at room temperature before use.

Step 1: Planar Surface Activation

1. Coating of discreet sections of the planar surface is possible via pipette spotting, otherwise cover the entirety of the planar surface to be activated with AnteoBind™ Biosensor.
2. Incubate for 30 minutes at room temperature. Continuous gentle agitation (e.g. orbital shaker at 25 rpm) is recommended but not required.

Note: Some planar surfaces may be activated in as little as 5 minutes. The optimal activation incubation time must be determined by the user.

3. Wash the planar surface with Type 1 water using the most appropriate method (refer to Special Operating Instructions) and then dry.

Note: Nitrogen and heat facilitated drying may be possible, AnteoTech recommends the users to determine the optimal drying conditions depending on the nature of the planar surface and the biomolecule of interest. Activated surfaces should be stored at 2-8 °C under low humidity conditions until required.

Step 2: Planar Surface Conjugation

1. Cover the entirety of the planar surface with biomolecule in diluent.

Note: The optimal biomolecule concentration and diluent must be determined by the user.

2. Incubate for 1 hour at room temperature. Continuous gentle agitation (e.g. orbital shaker at 25 rpm) is recommended but not required.

Note: Planar surfaces may be conjugated in as little as 10 minutes. The optimal conjugation incubation time must be determined by the user.

3. Wash the planar surface with wash buffer using the most appropriate method and then dry.

Note: The optimal wash buffer and drying conditions must be determined by the user.

Step 3: Planar Surface Blocking

1. Cover the entirety of the planar surface with Blocking Agent in diluent.

Note: The optimal Blocking Agent and the diluent may vary depending on the planar surface and biomolecule of interest and the intended application and must be determined by the user.

2. Incubate for 30 minutes at room temperature. Continuous gentle agitation (e.g. orbital shaker at 25 rpm) is recommended but not required.

Note: The optimal blocking incubation time may vary depending on the planar surface and biomolecule of interest and must be determined by the user.



3. Wash the planar surface with wash buffer using the most appropriate method and then dry.

Note: *The optimal wash buffer and drying conditions may vary depending on the planar surface and biomolecule of interest and must be determined by the user.*

4. Conjugated surfaces should be stored at 2-8 °C under low humidity conditions until required.



Troubleshooting

Issue	Possible Cause(s)	Recommendations
<i>Suboptimal performance of biomolecule</i>	Biomolecule concentration too high	Extend titration series lower if signal obtained has plateaued within the current range.
	Inappropriate blocking	Optimise Blocking Agent. AnteoTech recommends 10% (w/v) BSA prepared in <i>Coating Buffer</i> when working with antibodies or other high molecular weight proteins. Alternative blocking agents include but are not limited to, casein, fish skin gelatine, and synthetic blockers. Contact AnteoTech Technical Support for further details.
OR		
<i>Non-Specific Signal</i>	AnteoBind™ concentration not ideal for planar surface	Further dilution of AnteoBind™ using Type 1 water prior to surface activation.
	Activated surface stored under non-ideal conditions	Confirm AnteoBind™ stability on planar surface. Check that storage conditions were between 2 - 8° C under low humidity conditions (ideally with desiccant).