

Stable and Oriented Immobilization of *Strep-tag*[®] II Fusion Proteins on Biacore Sensor Chip CM5

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IBA Headquarters

IBA GmbH
Rudolf-Wissell-Str. 28
D-37079 Göttingen
Germany
Tel: +49 (0) 551-50672-0
Fax: +49 (0) 551-50672-181
info@iba-go.com
www.iba-go.com

IBA US Distribution Center

10748 Indian Head Industrial Blvd.
St. Louis, MO 63132
Tel. 1-877-IBA-GmbH (1-877-422-4624)
Fax 1-888-531-6813
info@iba-go.com
www.iba-go.com



Patents & Licensing

IBA patents, licensing and trademarks

Strep-tag[®] technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066; the tetracycline promoter based expression system is covered by US patent 5,849,576 and Strep-Tactin[®] is covered by US patent 6,103,493. Further patent applications are pending world-wide. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Expression or purification or other applications of above mentioned technologies for commercial use require a separate license from IBA. A license may be granted by IBA on a case-by-case basis, and is entirely at IBA's discretion. Please contact IBA for further information on licenses for commercial use.

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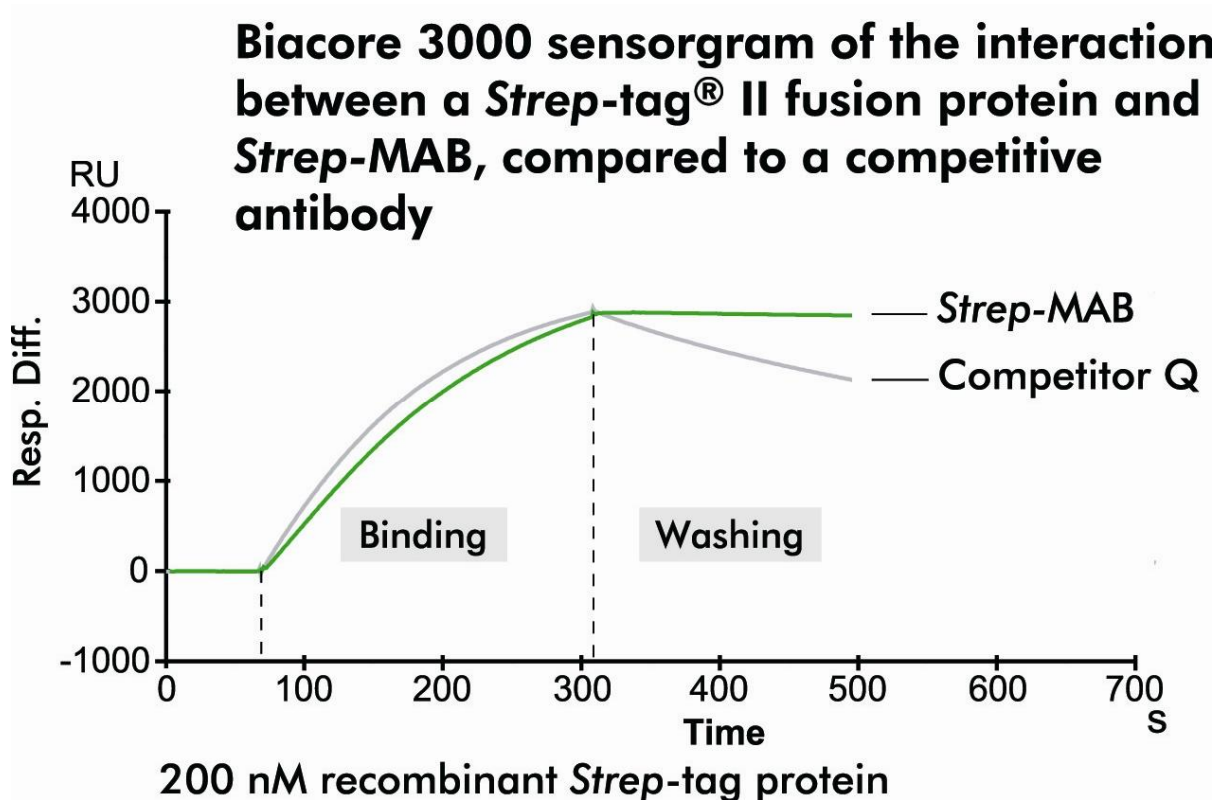
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StrepMAB-Immo™: High-affinity Strep-tag® II specific monoclonal antibody

StrepMAB-Immo™ is a murine, high-affinity Strep-tag® II specific monoclonal antibody which is especially suited for the stable, mild and oriented capturing of Strep-tag fusion proteins on solid phases. To realize this, the antibody can be immobilized on e.g. microplates, Biacore™ Sensor Chip CM5¹ or other biochips.

The almost irreversible binding is currently validated only for fusion proteins carrying a C-terminal Strep-tag with SerAla linker (NH₂-recombinant protein sequence-SA-WSHPQFEK-COOH; refer also to IBA's expression vectors at www.strep-tag.com).

For all other Strep-tag proteins we offer an alternative strategy by covalently linking the protein of interest to immobilized Strep-Tactin (please see protocol below).



During the washing phase, the recombinant Strep-tag protein of interest remains tightly bound to StrepMAB-Immo, while a significant amount of Strep-tag protein is washed off using the competitive antibody.

Preparation of *Strep*-tag fusion protein capturing sensor chip surface using *StrepMAB-Immo*

StrepMAB-Immo can be coupled to Biacore™ Sensor Chip CM5 surfaces using the standard amine coupling protocol.

Material required but not provided

- EDC: 0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide in water
- NHS: 0.1 M N-hydroxysuccinimide in water
- 1 M ethanolamine-HCl pH 8.5
- Running Buffer A: HBS-EP; 0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20 (BR-1001-88)

Sensor Chip CM5 (BR-1000-14) as well as reagents for amine coupling (BR-1000-50) are available from Biacore AB.

Protocol

- 1. Activate the surface: injection of EDC/NHS (1:1) at 10 μ l/min for 7 minutes.**
- 2. Immobilize *StrepMAB-Immo*: injection of 50 μ g/ml *StrepMAB-Immo* in 10 mM sodium acetate pH 5.0 at 10 μ l/min for 7 minutes.**
- 3. Deactivate excessive reactive groups: injection of ethanolamine at 10 μ l/min for 7 minutes.**

Using this procedure, typically around 15000 RU (approximately 15000 pg/mm², corresponding to 0.1 pmol) are generated due to immobilization of *StrepMAB-Immo*

The *StrepMAB-Immo* coated Sensor Chip CM5 is now ready to immobilize a *Strep*-tag fusion protein under native binding conditions (i.e. PBS pH 8). Bound proteins should be resistant to release under flow and are often found to remain stably bound even under low pH or chaotropic regeneration conditions so that surfaces should be stable and usable for several experiments (in case of transiently binding ligands). Regeneration with diluted NaOH will lead to denaturation of *StrepMAB-Immo*. Nonetheless, regeneration conditions need to be tested for every protein. For reproducible regeneration of the Sensor Chip CM5 proceed to the next section.

Preparation of a renewable capturing sensor chip surface for *Strep*-tag II fusion proteins using *Strep*MAB-Immo (A. Skerra, TU Munich)

Material required but not provided:

- EDC: 0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide in water
- NHS: 0.1 M N-hydroxysuccinimide in water
- 1 M ethanolamine-HCl pH 8.5
- Running Buffer B: PBS, 0.005 % v/v Surfactant P20
- Regeneration Buffer: 10 mM glycine/HCl pH 1.7

Sensor Chip CM5, rabbit anti mouse IgG (BR-1005-14) as well as reagents for amine coupling are available from Biacore AB.

Protocol

- 1. Activate the surface: injection of EDC/NHS (1:1) at 5 μ l/min for 7 minutes.**
- 2. Immobilize capture antibody: injection of rabbit anti mouse IgG (Biacore BR-1005-14) diluted to 50 μ g/ml in 10 mM sodium acetate pH 5.0 at 5 μ l/min for 7 minutes.**
- 3. Block reactive groups: injection of ethanolamine/HCl at 5 μ l/min for 7 minutes.**

Using this procedure, an additional resonance signal of typically around 6000 RU (approximately 6000 pg/mm², corresponding to 0.04 pmol protein) is obtained due to the covalent immobilization of the rabbit anti mouse IgG. The Sensor Chip CM5 is now ready for reversible capturing of the *Strep*MAB-Immo.

- 4. Apply 35 μ l *Strep*MAB-Immo (diluted to 25 μ g/ml in Running Buffer B) at 5 μ l/min for 7 minutes and wash with Running Buffer B.**

Usually, 500 RU are generated due to the non-covalent immobilization of *Strep*MAB-Immo. The chip is now ready to immobilize a *Strep*-tag II fusion protein and, subsequently, to record a sensorgram of the interaction between such a *Strep*-tag II fusion protein (e.g. a recombinant antibody fragment) and its ligand (e.g. an antigen). A series of sensorgrams at different ligand concentrations can be measured using the same chip. To this end the chip with the covalently attached capture antibody may be regenerated after each ligand-binding cycle by removing *Strep*MAB-Immo, the *Strep*-tag II fusion protein, and the bound ligand as set forth in the next step:

- 5. Wash with Regeneration Buffer at a flow rate of 5 μ l/min for 3 minutes.**

This procedure preserves the covalently attached rabbit anti mouse IgG and the chip is ready to record a new sensorgram by repeating step 4, followed by application of a different ligand or another ligand concentration.

Preparation of *Strep*-tag fusion protein capturing CM5 chip surface using *Strep*-Tactin

Strep-Tactin can be coupled to Biacore Sensor Chip CM5 chip surfaces using the standard amine coupling protocol. Immobilized *Strep*-Tactin can be activated by the addition of EDC/NHS without hampering its *Strep*-tag binding properties. Such activated immobilized *Strep*-Tactin can be used for mild and directed covalent immobilization of *Strep*-tag fusion proteins.

Material required but not provided

Sensor Chip CM5 as well as reagents for amine coupling are available from Biacore AB.

- EDC: 0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide in water
- NHS: 0.1 M N-hydroxysuccinimide in water
- 1 M ethanolamine-HCl pH 8.5
- Running buffer: HBS-EP; 0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20

Protocol

1. **Activate the surface: injection of EDC/NHS (1:1) at 10 μ l/min for 7 minutes.**
2. **Immobilize *Strep*-Tactin: injection of 50 μ g/ml *Strep*-Tactin in 10 mM sodium acetate pH 5.0 at 10 μ l/min for 7 minutes.**
3. **Deactivate excessive reactive groups: injection of ethanolamine at 10 μ l/min for 7 minutes.**

Using this procedure, typically around 5000 RU (approximately 5000 pg/mm², corresponding to 0.1 pmol protein) are generated due to immobilization of *Strep*-Tactin

Strep-tag proteins can be bound to this surface using native interaction conditions (i.e. PBS pH 8). However, binding is not stable and usually very transient and thus not suited for further analysis of interactions between the *Strep*-tag fusion protein and ligands. Therefore, a protocol was developed in cooperation with Biacore AB to activate *Strep*-Tactin with EDC/NHS without hampering its *Strep*-tag binding properties. If a *Strep*-tag fusion protein is now applied, it will be directed via the *Strep*-tag to the activated surface for mild and reliable covalent immobilization.

4. **Activate *Strep*-Tactin: injection of EDC/NHS (1:1) at 10 μ l/min for only 1 minute.**
5. **Immobilize *Strep*-tag fusion protein: injection of typically 20-50 μ g/ml ligand in PBS pH 8 at 10 μ l/min for 7 minutes.**

6. Deactivate excessive reactive groups: injection of ethanolamine at 10 μ l/min for 7 minutes.

The difference of the base line will indicate the RU caused by covalently immobilized *Strep*-tag fusion protein which can now be analyzed for its binding capabilities towards other ligands.

Related Products

Cat. No.	Product
2-1204-001	<i>Strep</i> -Tactin, lyophilized, 1 mg
2-1517-001	<i>Strep</i> MAB-Immo Antibody, lyophilized, 100 μ g

¹ For further information on Biacore™ applications refer to www.biacore.com/lifesciences
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