

# Stable and Oriented Immobilization of SA-Strep-tag<sup>®</sup> II Fusion Proteins on Biacore Sensor Chip CM5

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*Strep-tag*<sup>®</sup> technology for protein purification and detection is covered by worldwide patents (US5506121; DE4237113; JP3865792; UK2272698; FR9313066) and *Strep-Tactin*<sup>®</sup> is covered by US6103493 and patent applications in Europe. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Cloning, 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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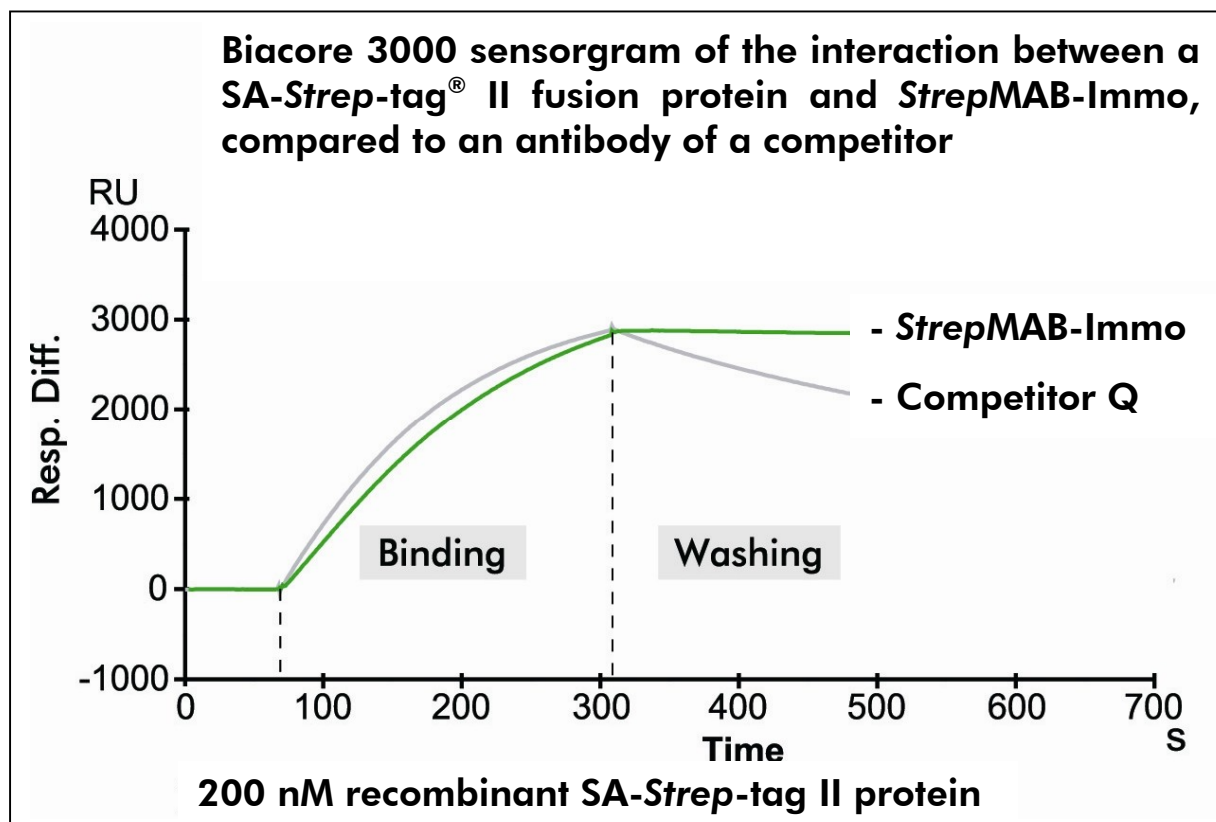
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## **StrepMAB-Immo™ : High-affinity SA-Strep-tag® II specific monoclonal antibody**

StrepMAB-Immo™ is a murine, high-affinity SA-Strep-tag® II specific monoclonal antibody which is especially suited for the stable, mild and oriented capturing of SA-Strep-tag II fusion proteins on solid phases. To realize this, the antibody can be immobilized on e.g. microplates, Biacore™ Sensor Chip CM5<sup>1</sup> or other biochips.

The almost irreversible binding is currently validated only for fusion proteins carrying a Strep-tag II with SerAla leader (SA-Strep-tag II: NH<sub>2</sub>SA-WSHPQFEK-COOH). SA-Strep-tag II may be fused to the N-terminal end or to the C-terminal end of the recombinant protein. In case of doubt, please refer also to our SA-Strep-tag II carrying Acceptor Vectors within the StarGate cloning system ([www.stargate-cloning.com](http://www.stargate-cloning.com)).

For all other Strep-tag proteins carrying a Strep-tag II without leading SerAla we recommend an alternative strategy by covalently linking the protein of interest to immobilized Strep-Tactin (please see protocol below).



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

# Preparation of a SA-Strep-tag II 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 µl/min for 7 minutes.**
- 2. Immobilize StrepMAB-Immo: injection of 50 µg/ml StrepMAB-Immo in 10 mM sodium acetate pH 5.0 at 10 µl/min for 7 minutes.**
- 3. Deactivate excessive reactive groups: injection of ethanolamine at 10 µl/min for 7 minutes.**

Using this procedure, typically around 15000 RU (approximately 15000 pg/mm<sup>2</sup>, 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 SA-Strep-tag II 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 SA-Strep-tag II fusion proteins using StrepMAB-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  $\mu$ l/min for 7 minutes.**
- 2. Immobilize capture antibody: injection of rabbit anti mouse IgG (Biacore BR-1005-14) diluted to 50  $\mu$ g/ml in 10 mM sodium acetate pH 5.0 at 5  $\mu$ l/min for 7 minutes.**
- 3. Block reactive groups: injection of ethanolamine/HCl at 5  $\mu$ l/min for 7 minutes.**

Using this procedure, an additional resonance signal of typically around 6000 RU (approximately 6000 pg/mm<sup>2</sup>, 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 StrepMAB-Immo.

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

Usually, 500 RU are generated due to the non-covalent immobilization of StrepMAB-Immo. The chip is now ready to immobilize a SA-Strep-tag II fusion protein and, subsequently, to record a sensorgram of the interaction between such a SA-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 StrepMAB-Immo, the SA-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  $\mu$ 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 II 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 II (= WSHPQFEK) binding properties. Such activated immobilized *Strep*-Tactin can be used for mild and directed covalent immobilization of *Strep*-tag II 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  $\mu$ l/min for 7 minutes.**
2. **Immobilize *Strep*-Tactin: injection of 50  $\mu$ g/ml *Strep*-Tactin in 10 mM sodium acetate pH 5.0 at 10  $\mu$ l/min for 7 minutes.**
3. **Deactivate excessive reactive groups: injection of ethanolamine at 10  $\mu$ l/min for 7 minutes.**

Using this procedure, typically around 5000 RU (approximately 5000 pg/mm<sup>2</sup>, corresponding to 0.1 pmol protein) are generated due to immobilization of *Strep*-Tactin

*Strep*-tag II 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 II 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 II binding properties. If a *Strep*-tag II fusion protein is now applied, it will be directed via the *Strep*-tag II to the activated surface for mild and reliable covalent immobilization.

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

**6. Deactivate excessive reactive groups: injection of ethanolamine at 10  $\mu$ l/min for 7 minutes.**

The difference of the base line will indicate the RU caused by covalently immobilized *Strep*-tag II 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 $\mu$ g

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<sup>1</sup> For further information on Biacore™ applications refer to [www.biacore.com/lifesciences](http://www.biacore.com/lifesciences)  
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