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Protocol

Coating of Biacore™ Sensor Chip CM5 with StrepMAB-Immo

1. INTRODUCTION

StrepMAB-Immo is a murine, high-affinity monoclonal antibody which is especially suited for stable, mild, and oriented capturing of Strep-tag®II and Twin-Strep-tag® fusion proteins on solid phases. That ability allows the coating of SPR sensor chips like Biacore™ Sensor Chip CM5 whereby binding affinities and/or kinetics of captured Strep-tag® fusion protein to a specific analyte can be determined.
The nearly irreversible binding is validated for fusion proteins carrying a C- or N-terminal Strep-tag®II providing an N-terminal extension by a SerAla linker (recombinant protein-SA-WSHPQFEK or SA-WSHPQFEK-recombinant protein). Proteins containing the Strep-tag®II with N-terminal sequences other than SerAla are bound with reduced affinity in the cases tested so far. However, the Twin-Strep-tag® always contains the SerAla linker (WSHPQFEK-GGGS-GGGS-GGSA-WSHPQFEK).

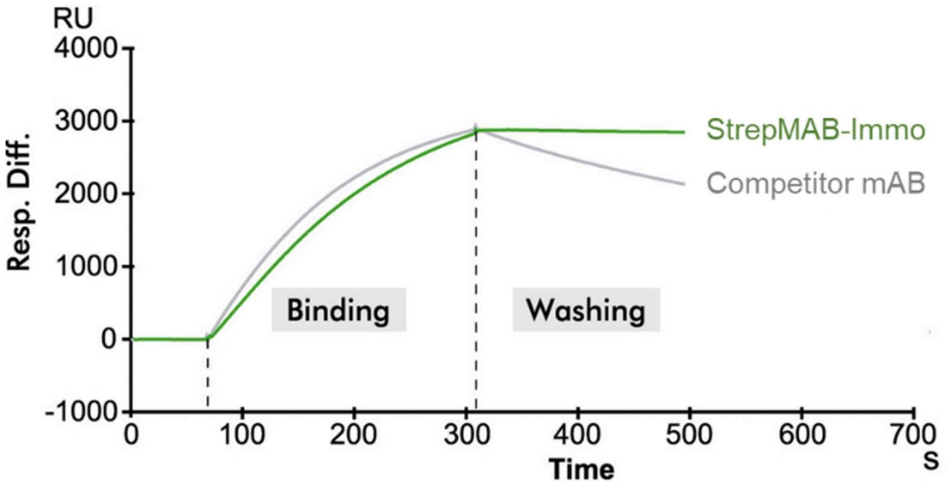

Fig.1: Biacore 3000 sensorgram of the interaction between a Strep-tag®II fusion protein with SA linker and StrepMAB-Immo, compared to an antibody of a competitor (competitor mAB). 200 nM recombinant Strep-tag®II fusion protein was injected for capture. During the washing phase, the recombinant Strep-tag®II fusion protein with SA linker remains tightly bound to StrepMAB-Immo, while a significant amount of the same protein is washed off using the competitive antibody.

2. REQUIRED PRODUCTS

Besides StrepMAB-Immo (Cat. No. 2-1517-001), all necessary reagents for coating of Biacore™ Sensor Chip CM5 can be received by Cytiva.

Product	Comment
StrepMAB-Immo	Reconstituted with 200 µl water to obtain a stock solution with 0.5 mg/ml in PBS (2.5 mM KCl, 8 mM Na2HPO4, 200 mM NaCl, 1.5 mM KH2PO4, pH 7.4). Dilute in 10 mM sodium acetate pH 5.0 prior to application.
EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide)	0.4 M in water
NHS (N-hydroxysuccinimide)	0.1 M in water
Ethanolamine-HCl	1 M, pH 8.5
Running Buffer A (HBS-EP)	10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20
Biacore™ Sensor Chip CM5	-

3. PROTOCOL

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

2.1 Activate the surface: injection of EDC/NHS (1:1) at 10 µl/min for 7 minutes.

2.2 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.

2.3 Wash with Running Buffer A until baseline is stable.

2.4 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^2, corresponding to 0.1 pmol) are generated due to immobilization of StrepMAB-Immo.

The StrepMAB-Immo coated Biacore™ Sensor Chip CM5 is now ready to immobilize a Strep-tag® fusion protein under native binding conditions (e.g., PBS pH 8.0). Regeneration with diluted NaOH will lead to denaturation of StrepMAB-Immo. Nonetheless, regeneration conditions need to be tested for every protein.

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If you have any questions, please contact

strep-tag@iba-lifesciences.com

We are here to help!

IBA Lifesciences GmbH · Rudolf-Wissell-Str. 28 · 37079 Goettingen · Germany · Phone + 49 551 50672-0 · info@iba-lifesciences.com