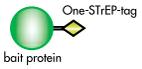
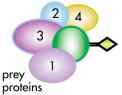


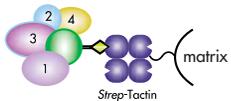
1 Express bait protein in target cells



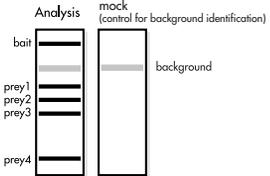
2 Complex formation



3 Isolate protein complex on Strep-Tactin column



4 SDS-PAGE



Excise additional bands not present in control. Digest with Trypsin. Analyse by mass spectrometry and bioinformatics.

5



Identification of prey 1-4

Isolate protein complexes in one STEP!

- Minimal background
- Reduced sample dilution
- Fast protocol
- Isolates transiently and weakly interacting proteins

- Suitable to identify several binding partners/co-factors at once
- Positive control system included!

The new One-STrEP™ system is an extremely fast and efficient method to identify protein complexes. While other systems require tedious optimization because of high background or two successive purification steps, the One-STrEP system requires

one STEP only to isolate intact protein complexes. In this system *Strep*-Tactin columns are used for fast protein purification under physiological conditions with low background enabling even the isolation of weakly associated binding partners.

One-STrEP Procedure

Cells are transfected with the plasmid coding for a bait protein fused to the One-STrEP-tag*. In the desired state of growth, cells are lysed and the bait together with bound proteins (preys) is isolated using a *Strep*-Tactin

column. Proteins are resolved on a SDS-gel and specifically bound proteins (preys not appearing in the control) can be identified by mass spectrometry or verified by Western blot using specific antibodies.

One-STrEP Starter Kit

The Kit includes the plasmid pEXPR-IBA103** for bait cloning and expression as well as various *Strep*-Tactin columns and buffers for the isolation of protein complexes in different scales. Also, it contains a One-STrEP-tag monoclonal antibody conjugated to HRP for detection of the bait. As a control for the entire procedure, a control plasmid

encoding a bait (PR65) and antibodies for a specifically binding prey (PP2Ac) are included as well. Both PR65 and PP2Ac are subunits of the phosphatase 2A protein complex, which is present in most mammalian cells. A positive detection of the prey in the eluate proves the successful isolation of an intact protein complex [Junttila et al., 2004].

Order information

Cat.no.	description	amount
2-1109-000	One-STrEP Starter Kit	1 Kit
2-1110-000	One-STrEP Follow-up Kit (without controls)	1 Kit

Components of the One-STrEP Starter Kit

Components	amount
Gravity flow <i>Strep</i> -Tactin® Superflow® columns	5 x 1 ml
Gravity flow <i>Strep</i> -Tactin® Superflow® mini columns	5 x 0.2 ml
pEXPR-IBA103 mammalian expression vector	5 µg
Control vector with PR65 insert (bait)	5 µg
One-STrEP-tag specific monoclonal antibody, HRP conjugate	for 15 Western blots (50 µl)
PP2Ac affinity-purified goat polyclonal antibody	for 5 Western blots (38 µl)
Buffer L (lysis)	150 ml
Buffer W (washing)	120 ml
Buffer BE (elution)	25 ml
Manual	1

* One-STrEP-tag is a modification of our successful *Strep*-tag® II. The tag has been adapted to higher detergent compatibility, which is important for optimizing binding and washing conditions for protein:protein interactions.

** The pEXPR-IBA103 plasmid included in the One-STrEP-Kit is based on pEXPR-IBA3 (see page 3 of this newsletter) but contains the new One-STrEP-tag instead of *Strep*-tag II.

Junttila MR, Saarinen S, Schmidt T, Kast J, Westermarck J (2004) Single-step *Strep*-tag® purification for the isolation and identification of protein complexes from mammalian cells. *Proteomics*, in press.

IBA product portfolio



Request further information at

www.iba-bioTAGnology.com

and subscribe to our e-newsletter at http://www.iba-bioTAGnology.com/abo/reg_fr.html

IBA GmbH | Rudolf-Wissell-Straße 28 | 37079 Göttingen | Germany
Phone +49(0)551 5 06 72-0 | Fax +49(0)551 5 06 72-181 | info@iba-go.com | www.iba-go.com

IBA US Distribution Center | 1508 South Grand Blvd. * St. Louis, MO 63104 | USA
Tel. 1-877-IBA-GmbH (1-877-422-4624) | Fax 1-888-531-6813 | info@iba-go.com | www.iba-go.com

TAGnologies™, *Strep-tag*®, *Strep-Tactin*® and One-STrEP™ are trademarks of IBA GmbH.
Superflow® is a trademark of Sterogene Bioseparations Inc. Products sold for research use only.