One-STrEP Application Note

One-STrEP-tag for protein:protein-interaction analysis

Johansen et al., 2008

Protein:protein-interactions (PPI) govern almost all important processes in living organisms. Thus, their rapid and accurate determination and investigation is a major challenge in life sciences.

The One-STrEP-tag is a reliable tool to isolate protein complexes at high purity without losing transient binders. The advantages of the One-STrEP system in comparison to the commonly used TAP system are that only one tag is needed and the isolation of the protein complex components is carried out in one step. An One-STrEP-tagged bait protein is expressed in the target cell and the cell lysate containing the bait and the putatively interacting preys is subjected to tag-based affinity chromatography on *Strep*-Tactin®. The isolated protein complexes are analyzed by SDS-PAGE and potential preys are identified by mass spectrometry (Rigaut et al., 1999). In several cases the One-STrEP-tag was already successfully applied to isolate and identify bait proteins and its interaction partners (see below for references). Johansen et al., 2008,

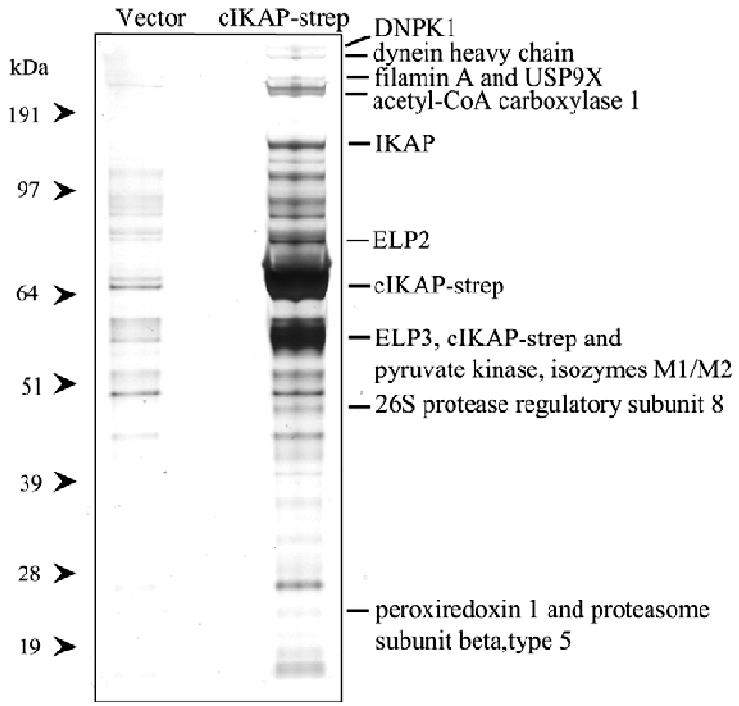

Figure 1: IKAP co-purifies with various cytosolic proteins.

One-STrEP-tag purification. HEK293 cells transiently transfected with either empty vector or cIKAP-strep were harvested 2 days after transfection and cytosolic extracts were prepared as described in the Materials and Methods. Purification was performed according to the manufacture´s instructions (IBA). Eluates were concentrated and run on 10% SDS-PAGE. Silver staining of the purified proteins. The indicated proteins were identified by MALDI-TOF-MS. DNPK1, DNA-dependent protein kinase; USP9X, ubiquitin-specific processing protease.

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screened for interaction partners of the IκB-kinase-associated protein, IKAP, which is involved in the neurodegenerative disease familial dysautonomia. "Previous studies of proteins that might associate with IKAP revealed no candidates that would fit with both its cytosolic localization and function in cell migration. Therefore we decided to use the One-STrEP-tag, since it seems to be an excellent tag for the identification of even weakly-binding interactors" (Tuula Kallunki, personal comment). The One-STrEP-tagged C-terminus of IKAP (cIKAP-strep) was used as bait and a total of 15 proteins were identified to associate specifically with cIKAP-strep (Figure 1). Two of the potential interactors (filamin A and dynein heavy chain) had been linked to neuronal migration in previous studies, what coincides with one of the functions IKAP is involved. Further analysis of IKAP and filamin A revealed their co-localization and verified their association. This example is demonstrating the effectiveness of the One-STrEP system purifying protein complexes in combination with protein identification via MS and adjacent functional analysis.

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