GFP as affinity tag for Immunoprecipitations

Fluorescent proteins are widely used to study protein localization and dynamics. They have redefined fluorescence microscopy because they are non-toxic and less harmful when illuminated in live cells as compared to chemical fluorescent dyes.

Data generated from microscopic studies alone are not sufficient to acquire comprehensive information about a protein. Necessary additional information on DNA binding, enzymatic activity, and complex formation can be obtained with various methods including chromatin Immunoprecipitation (ChIP) and affinity purification. The challenge for researchers is to select a high-quality antibody for these studies: Often a specific antibody for their protein of interest is not available, making it necessary to fuse the protein of interest to specific epitopes where antibodies are available. The most popular epitopes include c-Myc, FLAG and GST. Interestingly GFP and RFP, the most widely used labeling tags in cell biology, are rarely used for biochemical analyses, although researchers need to go through additional cloning and transformation steps, when they already have cells expressing their protein of interest fused to a fluorescent protein. This may be due in part to the limited availability of good antibodies against GFP and RFP.
This document compares existing conventional antibodies against GFP/RFP in biochemical studies with the GFP-/RFP-Trap®, a ready-to-use pulldown reagent derived from alpaca.

2. Experimental procedures

Immunoprecipitation

1 x 10^6–1 x 10^7 HEK 293T cells either mock-treated or transiently transfected with expression vectors coding for GFP were homogenized in 200 µl of lysis buffer (20 mM Tris/HCl, pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 2 mM PMSF, 0.5% Nonidet P-40). After a centrifugation step (10 min at 20,000 x *g* at 4 °C) the supernatant was adjusted with dilution buffer (20 mM Tris/HCl, pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 2 mM PMSF) to 1 ml. 20 µl (2%) were added to SDS-containing sample buffer (referred to as input). 25µl GFP-Trap® or 2 µg of anti-GFP antibodies (clones 7.1 and 13.1, Roche Diagnostics) were added and incubated for 5– 60 min on an end-over-end rotor at 4°C. For pulldown of immunocomplexes with antibodies 25 µl of an equilibrated mixture of protein A/G-Sephaose (Amersham Biosciences) were added, and incubation continued for 60 min. After a centrifugation step (2 min at 5000 x *g* at 4 °C) supernatant was removed, and 2% was used for SDS-PAGE (referred to as flow-through). The bead pellet was washed two times in 1 ml of dilution buffer containing 300 mM NaCl. After the last washing step the beads were resuspended in 2x SDS-containing sample buffer and boiled for 10 min at 95 °C.

3. Workflow comparison

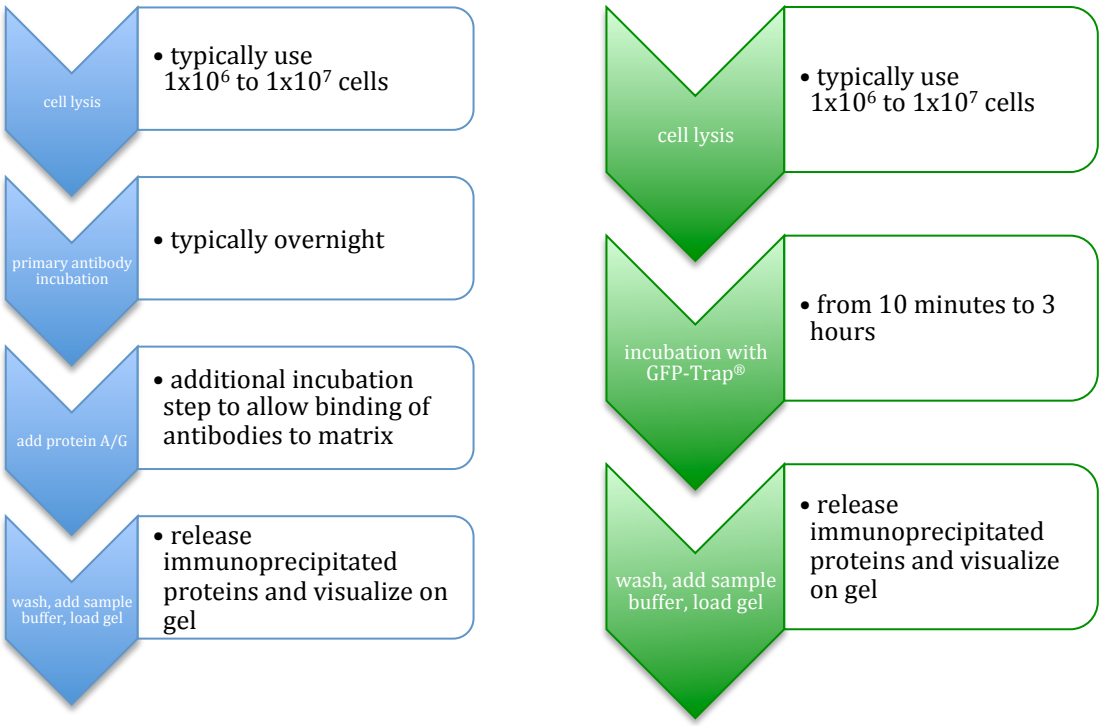

Figure 1: Workflow comparison for Immunoprecipitations with conventional antibodies (left) and with alpaca based Nano-Traps (right). The high specificity allows for significantly shorter incubation times.

4. Typical Immunoprecipiation results

GFP-Trap® and anti-GFP antibodies were added to protein extracts of HEK 293T cells expressing GFP. Precipitated proteins were separated by SDS-PAGE and visualized by Coomassie Blue or immunoblot analysis. GFP-Trap® is covalently coupled to sepharose beads, hence there are no contaminating additional bands visible (figure 2). Analysis of the precipitated proteins with conventional antibodies, the typical low and heavy chain bands can be seen at approximately 25, 50 and 60 kDa respectively.

In addition, the results show a quantitative GFP depletion from the flow-through fraction with GFP-Trap®, whereas the flow-through of IPs with conventional antibodies still shows significant amounts of GFP.

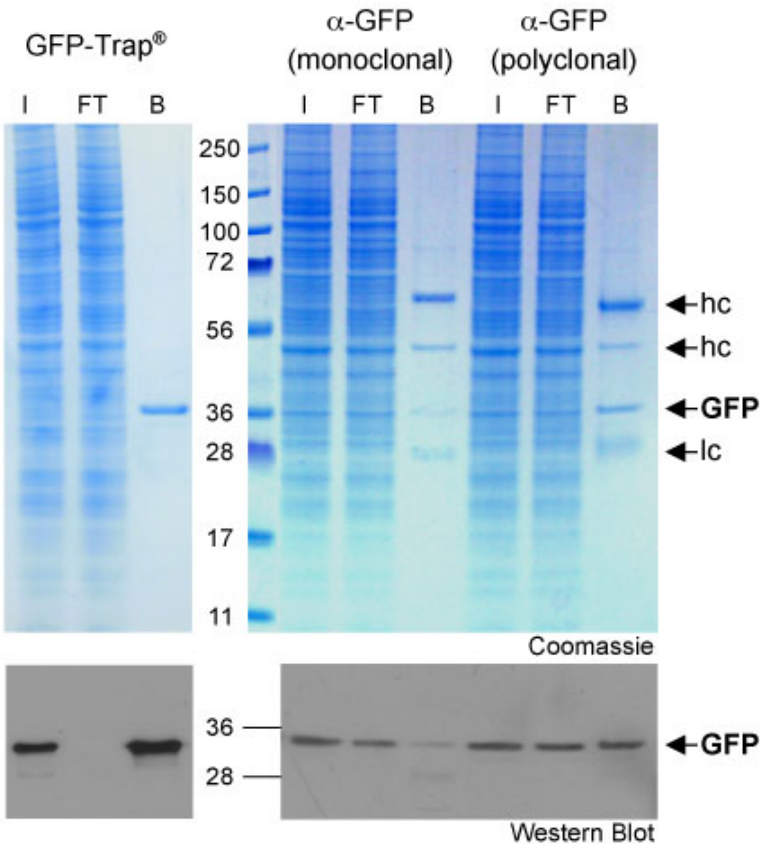

Figure 2:
Immunoprecipiation of GFP from cell extracts. Protein extracts of GFP-producing HEK 293T cells were subjected to immunoprecipitation with GFP-Trap®, mono-, or polyclonal anti-GFP antibodies. Aliquots of input (I), flow-through (FT), and bound fraction (B) were separated by SDS-PAGE and visualized by Coomassie Blue (top) or by immunoblot analysis (bottom). Precipitated GFP, denatured heavy (hc) and light chains (lc) of the IgGs are marked by arrows.

5. Discussion

One challenge of the postgenomics era is the effective integration of genetic, biochemical, and cell biological data. This integration has in part been impeded by the simple fact that different protein tags are used for different applications. The results presented here show, that fluorescent proteins can be efficiently used as affinity tag for biochemical studies of fusion proteins. The advantages are obvious: In addition to saving the time to create additional fusion-proteins and cell lines, researchers can now do biochemical studies of the same protein they use for their imaging experiments.

GFP as affinity tag: GFP-Trap®

For the complete cell biological and biochemical characterization of proteins different fusions are no longer a necessity. GFP-Trap® offers scientists an easy way for biochemical analyses of GFP fusion proteins. Based on the antigen binding domain of alpaca heavy-chain antibodies, GFP-Trap® excels with high affinity. Masking of target proteins by heavy or light chains is not an issue, since GFP-Trap® is only 13 kDa in size. Thus the precipitates can be directly used in downstream applications like mass spectroscopy.

Ready-to-use coupled to monovalent matrices (Agarose or magnetic particles), GFP-Trap® allows immunoprecipitations of GFP fusion proteins and their interacting partners in less than 30 minutes.

References

Rothbauer, U., Zolghadr, K., Muyldermans, S., Schepers, A., Cardoso, M. C., Leonhardt, H. (2008): A Versatile Nanotrap for Biochemical and Functional Studies with Fluorescent Fusion Proteins, Mol Cell Proteomics. 2008 Feb;7(2):282-9. Epub 2007 Oct 21