Immunoprecipitation of Myc-tagged proteins

Benefits from the superior performance of Myc-Trap compared to conventional Myc antibodies

Preamble

This document provides a comparison of Myc-Trap® and traditional anti-Myc antibodies when applied for immunoprecipitation. Furthermore we describe how you benefit from the Myc-Trap in your experiments.

Introduction

The Myc epitope is a popular and widely used peptide tag. The Myc tag can be used to detect recombinant proteins in bacteria, yeast, insect and mammalian cell systems. Its short peptide sequence is N-EQKLISEEDL-C and has a size of about 1.2 kDa. The Myc tag is recognized by Myc-Trap and conventional anti-Myc antibodies at the N-terminus, C-terminus, or internal sites of the fusion protein (NB: not all traditional anti-Myc antibodies detect the tag at all 3 sites). For biochemical analysis including mass spectrometry and enzyme activity measurements these Myc tagged proteins and their interacting factors can be isolated by immunoprecipitation. ChromoTek's Myc-Trap increases the efficiency and accelerates your experiments. This is because it utilizes a small recombinant alpaca single chain antibody, a so-called "nanobody", covalently coupled to the surface of agarose or magnetic agarose beads.

Myc-Trap vs. anti-Myc antibody: Technology

Conventional antibodies are powerful tools in life science research. However, they have a large and complex structure, two heavy and two light chains, which can be troublesome in certain applications. *Camelidae*, i.e. alpacas, llamas, camels and dromedaries, possess a second type of antibodies. These are called heavy chain antibodies (hcAbs), see figure 1. HcAbs are devoid of light chains and bind their antigen via a single variable domain (V_HH). The V_HH domain is also known as "nanobody". They have excellent binding properties and can be produced at constant high quality without batch-to-batch variations. The ChromoTek Myc-Trap is ready to use, because it is coupled to agarose or magnetic agarose beads.

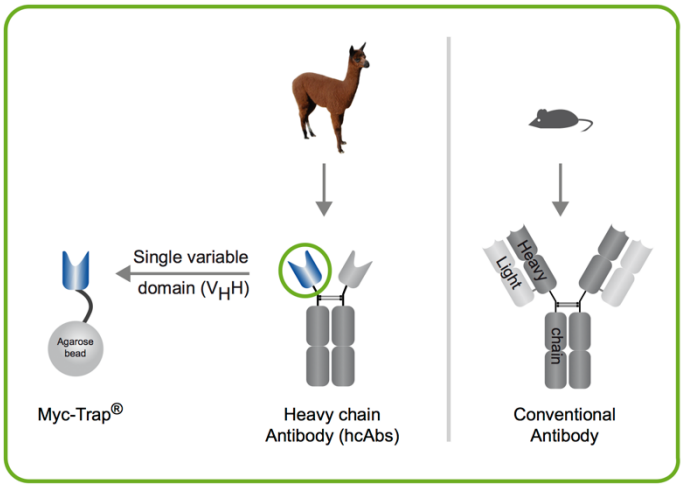

Figure 1: *The ChromoTek Myc-Trap consists of an anti-Myc V_HH coupled to agarose or magnetic agarose beads. Unlike traditional antibodies the Myc-Trap's V_HH does not contain heavy and light chains that may interfere with downstream applications.*

Myc-Trap vs anti-Myc-antibodies: Reduced background in immunoprecipitation

We compared the Myc-Trap (Figure 2, left) and anti-Myc antibodies coupled to Protein A/G (Figure 2, right). Input (I), non-bound (FT) and bound (B) fractions processed for IP with were separated by SDS-PAGE followed by Coomassie staining and Western Blotting. Myc tagged protein was purified using both approaches.
The bound fraction of the Myc-Trap comprised only the purified Myc-fusion protein of interest. In contrast, the bound fraction of the conventional Myc antibodies contained two additional strong bands besides the purified protein of interest. These bands -at approximately 50 kDa and 25 kDa- were contaminating heavy and light chains from the conventional anti-Myc antibody used for the immunoprecipitation. This may be a serious problem if you want to detect Myc tagged proteins or interacting factors of similar size.

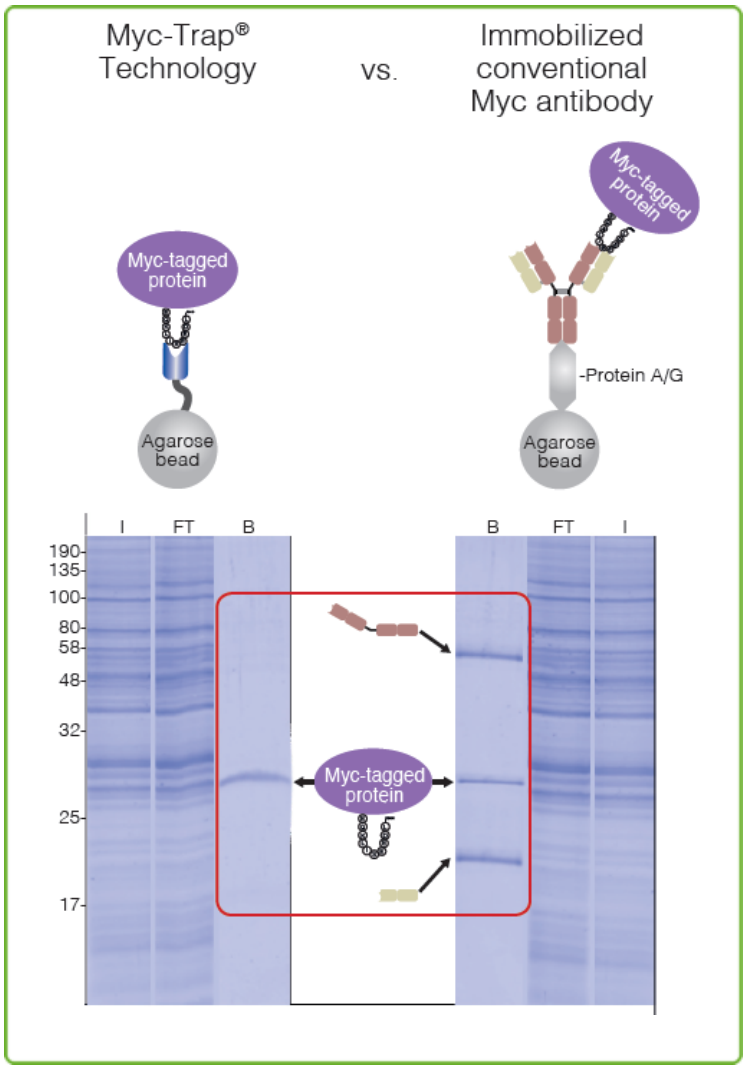

***Figure 2**: Immunoprecipitation of a Myc-fusion protein from cell extracts. Protein extracts of Myc-producing HEK 293T cells were subjected to immunoprecipitation with Myc-Trap or monoclonal anti-Myc antibody. Input (I), flow-through (FT), and bound fractions (B) were separated by SDS-PAGE and visualized by Coomassie Blue staining (top) or by immunoblot analysis (bottom). Precipitated Myc (violet ellipse), denatured heavy (brown cartoon) and light chains (beige cartoon) of the IgG are marked by arrows.*

Myc-Trap vs. anti-Myc-antibodies: Save time

The Myc-Trap allows for faster conduction of experiments than traditional antibodies. Here is a workflow comparison:
Myc-Trap: As shown in figure 3 left, after addition of cell lysate to the ChromoTek Myc-Trap the immunoprecipitation of the Myc-tagged protein takes place within two hours, including gentle elution with Myc-peptide.
Traditional Myc-antibodies: As you can see in figure 3 right, the immunoprecipitation with classical IgGs also last two hours; dependent on the affinity of the antibody it may actually be longer. Plus, in a second step, the Myc-fusion protein antibody complex may have to be coupled to protein A/G beads, which takes another 1-2 hours. This makes the entire workflow to last typically more than 3 hours or even longer.

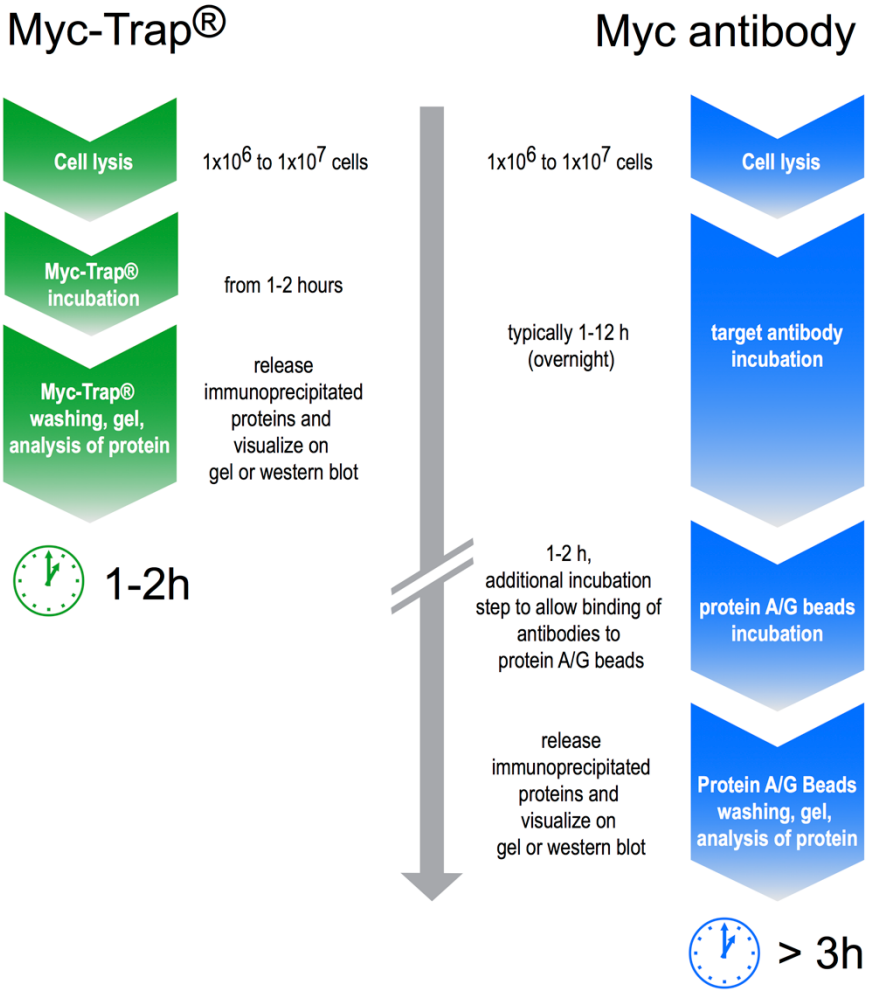

***Figure 3**: Workflow comparison between immunoprecipitations with alpaca Nanobody derived Myc-Trap (left) and with traditional anti-Myc IgG antibodies (right).*

Myc-Trap vs. anti-Myc-antibodies: Overview and summary

Myc-Trap	anti-Myc antibody
⊕ **No** heavy & light antibody chains in your downstream application	⊖ Contaminating heavy & light antibody chains may appear in your downstream application
⊕ Ready to use	⊖ Additional incubation step for binding of antibodies to protein A/G beads
⊕ High binding affinity	± High binding affinity may depend on actual antibody or polyclonal used
⊕ High reproducibility due to recombinant expression with very low batch to batch variation	± Reproducibility of monoclonals may depend on batch – polyclonals are different
⊕ Gentle elution with Myc-peptide	⊕ Gentle elution with Myc-peptide
⊖ Not suitable as detection antibody in Western Blot	⊕ Can generally be used as detection antibody in Western Blot

Further reading:

The Myc-Trap has been recently launched in mid-2015 and we have started to collect publications that our customers have published successfully using the ChromoTek Myc-Trap in a web-based database. We are frequently updating this database. You can filter your selection by organism, and type of experiment at our website www.chromotek.com/references, to find relevant publications relevant for your studies. You can also find a section on frequently asked questions at www.chromotek.com.