info@chromotek.com
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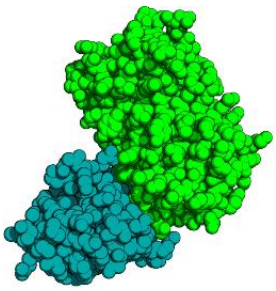
Application note

How to elute bound GFP-fusion protein from GFP-Trap

Content

1. Introduction

The ChromoTek GFP-Trap® is an affinity resin for immunoprecipitation (IP) and Co-IP of GFP-fusion proteins from cell extracts of various organisms. GFP-Trap consists of a 1 pM high affinity anti-GFP Nanobody/ VHH that is coupled to agarose or magnetic agarose beads or magnetic particles M-270. This high affinity enables very effective pulldowns of even low expressed GFP-fusion proteins but also requires special protocols for elution of bound GFP-fusion proteins if they are not analyzed on-bead.



anti-GFP Nanobody:GFP complex

2. Analysis options for bound GFP-fusion protein including on-bead and elution methods

There are multiple options for the subsequent analysis of bound GFP-fusion proteins and their interacting proteins. You may want to consider on-bead methods as alternative to elution:

- On-bead digestion for mass spectrometry (MS) analysis
- On-bead enzymatic assay

If the protocol requires elution for subsequent analysis, GFP-fusion proteins can be eluted from GFP-Trap by below methods:

- Elution with SDS-sample buffer for SDS-PAGE and/or Western blotting
  It is recommended to analyze all IP results by immunoblotting of input, flow through, and bound/elution fractions.
- Acidic elution for multiple biochemical assays of functional active proteins
- Enzymatic cleavage as a gentle elution option

3. On-bead methods as alternative to elution

3.1. On-bead digestion for mass spectrometry (MS) analysis

When sample preparation for MS analysis is conducted on-bead, it is more likely to retain all interaction partners of the protein of interest for MS analysis, whereas the elution from GFP-Trap may cause loss of sample, e.g. by incomplete elution. Trypsin digestion of the 14 kDa GFP-Nanobody/ V_HH from the GFP-Trap results in only 4-5 peptides. This small number of V_HH peptides does not add complexity to the MS analysis of the total protein sample. An on-bead digestion protocol is also used in the iST GFP-Trap Kit for processing GFP-fusion proteins and their interacting partners for MS analysis including GFP-fusion proteins. Next to GFP-Trap Agarose, the kit contains the PreOmics iST buffers and cartridges required for proteomic sample preparation.

Applications Note On-bead digestion protocol
Smits, A.H. (2013) Nucleic Acids Research, 41 (1), e28, doi: 10.1093/nar/gks941
Lipinszki, Z., et al (2014) Methods Mol Biol., 1170, 571-88, doi: 10.1007/978-1-4939-0888-2_33.
Turriziani, B. et al., (2014) Biology, 3(2), 320-332, doi: 10.3390/biology3020320
Kloet, S.L., et al. (2016) Nat Struct Mol Biol. 2016, 23(7), 682–690, doi: 10.1038/nsmb.3248

3.2. On-bead enzymatic assay

On-bead enzymatic assays are conducted, when the GFP-tagged enzyme is immobilized on the GFP-Trap. This is particularly important for pH sensitive proteins that can't be eluted by pH shift or other means. Generally, because the enzyme is immobilized via the GFP-tag, its enzymatic activity may not be compromised by the immunocapture.

Applications Note Enzyme activity assay
Yan, M. et al. (2015) Scientific Reports, 5, 10449, doi: 10.1038/srep10449

4. Elution options & protocols

Depending on the subsequent application, GFP-fusion proteins can be eluted from the beads by different elution methods. These methods provide an efficient elution from the GFP-Trap with only small amounts of GFP-fusion protein left on the beads.

Perform cell lysis and immunoprecipitation according to the standard GFP-Trap protocol. After washing the beads and removing the supernatant, continue with one of the elution methods below.

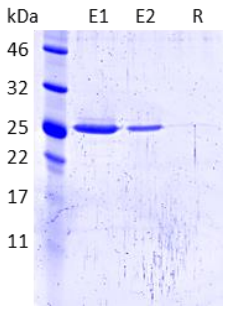
4.1. Elution with SDS-sample buffer (Laemmli) for SDS/PAGE and/or Western blotting

- Resuspend beads in 80 µL 2x SDS-sample buffer.
- Boil beads for 5 min at 95°C to dissociate immunocomplexes from beads.
- *GFP-Trap Agarose*: Sediment beads for 2 min at 2.500x g and room temperature. Collect supernatant and analyze by SDS-PAGE.
  GFP-Trap Magnetic Agarose and Magnetic Particles M-270: Magnetically separate beads until supernatant is clear. Collect supernatant and analyze by SDS-PAGE.

4.2. Acidic elution with glycine elution buffer (low pH)

- Remove the remaining supernatant.
- Add 50-100 µL Glycine elution buffer and constantly pipette up and down for 30-60 sec at +4°C or room temperature.
- *GFP-Trap Agarose*: Sediment beads by centrifugation at 2,500x g for 2 min at +4°C.
  GFP-Trap Magnetic Agarose and Magnetic Particles M-270: Magnetically separate beads until supernatant is clear.
- Transfer the supernatant to a new tube.
- Immediately neutralize the eluate fraction with 5-10 µL Neutralization buffer.
- Repeat this step at least once to increase elution efficiency.

Wash/Dilution buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Glycine elution buffer	200 mM glycine pH 2.5
Neutralization buffer	1 M Tris pH 10.4
2x SDS-sample buffer (Laemmli)	120 mM Tris/Cl pH 6.8; 20% glycerol; 4% SDS, 0.04% bromophenol blue; 10% β-mercaptoethanol



Immunoprecipitation of EGFP (28.0 kDa) using GFP-Trap Agarose and elution with Glycine elution buffer. Coomassie blue stained SDS-PAGE. Elution fractions E1 & E2. Residual fraction R was finally prepared by boiling beads in 2x SDS-sample buffer.

4.3. Enzymatic cleavage as a gentle elution option using TEV protease

The gentlest elution option is the cleavage of the protein of interest from the GFP using a specific protease like TEV protease from tobacco etch virus. This highly sequence-specific cysteine protease recognizes the amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser) and cleaves between the Gln and Gly/Ser. It does, however, cleave also some related sequences. You may check whether your protein of interest contains these amino acid sequences; the GFP Nanobody/ V_HH of GFP-Trap does not comprise these sequences. The TEV cleavage site needs to be cloned between your protein of interest and GFP at an appropriate position. After cleavage, TEV protease may be separated from the protein of interest by means of affinity purification, e.g. Ni-NTA if the protease is His-tagged.

Orlowska, K.P. et al. (2013) Nucleic Acids Research, 1–13, doi: 10.1093/nar/gkt650
Kehrein, K. et al. (2015) Cell Reports 10, 843-853, doi: 10.1016/j.celrep.2015.01.012

5. Related products for the analysis of GFP-fusion proteins

GFP Toolbox	Product Code
GFP-Trap® Agarose	gta-10; -20; -100
GFP-Trap® Agarose Kit	gtak-20
GFP-Trap® Magnetic Agarose	gtma-10; -20; -100
GFP-Trap® Magnetic Agarose Kit	gtmak-20
GFP-Trap® Magnetic Particles M-270	gtd-10; -20; -100
GFP-Trap® Magnetic Particles M-270	gtdk-20
iST GFP-Trap Kit for IP/MS	gtak-iST-8
Binding Control Agarose	bab-20
Binding Control Magnetic Agarose	bmab-20
Spin Columns	sct-10; -20; -50
GFP-Trap® Multiwell Plate	gtp-96
GFP VHH, recombinant binding protein	gt-250
GFP VHH, biotinylated recombinant binding protein	gtb-250
EGFP, recombinant purified protein	EGFP-250
GFP antibody [3H9] (rat monoclonal)	3h9-20; -100
GFP antibody [PABG1] (rabbit polyclonal)	PABG1-20; -100
Alpaca anti-rabbit IgG, recombinant VHH, Alexa Fluor® 488 Alpaca anti-rabbit IgG, recombinant VHH, Alexa Fluor® 568 Alpaca anti-rabbit IgG, recombinant VHH, Alexa Fluor® 647	srbAF488-1-10; -100 srbAF568-1-10; -100 srbAF647-1-10; -100
GFP-Booster Alexa Fluor® 488 GFP-Booster Alexa Fluor® 568 GFP-Booster Alexa Fluor® 647 GFP-Booster ATTO488 GFP-Booster ATTO594 GFP-Booster ATTO647N	gba2AF488-10; -50 gba2AF568-10; -50 gba2AF647-10; -50 gba488-10; -100 gba594-10; -100 gba647n-10; -100

For product details, information, and ordering visit www.chromotek.com.

Contact: support@chromotek.com

ChromoTek GmbH
Am Klopferspitz 19
82152 Planegg-Martinsried, Germany
phone: +49 89 124 148 80
fax: +49 89 124 148 811

ChromoTek Inc.
62-64 Enter Ln
Islandia, NY 11749, USA
phone: 631 501 1058
fax: 631 501 1060

ChromoTek introduced its GFP-Trap in 2008. Today, scientists apply GFP-Trap in a multitude of applications for GFP-tagged proteins because of its outstanding binding performance. With more than 1,600 publications, the GFP-Trap is the gold standard for immunoprecipitation of GFP-tagged proteins. For research use only.