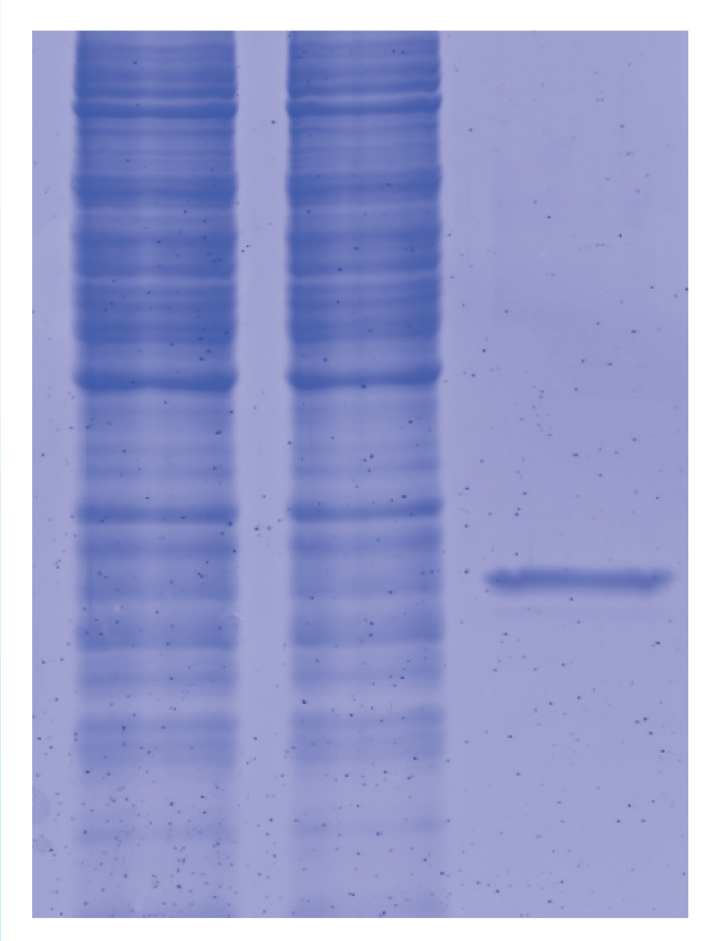
proteintech®

chromotek® Nano-Traps for superior immunoprecipitation



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What is a Nano-Trap?

Camelids such as camels, llamas, and alpacas possess an immune repertoire of three subclass IgG antibodies: IgG1, IgG2, and IgG3. IgG1 is a conventional IgG composed of two heavy chains and two light chains. IgG2 and IgG3 are heavy-chain-only IgG antibodies (HCAbs) that can be distinguished by their hinge regions. These HCAbs lack the CH1 domain of the heavy chain and are devoid of any light chain.

The binding domain of a heavy-chain-only IgG is called a Nanobody or V_HH. Nanobodies have excellent binding properties and can be recombinantly expressed at constant high quality with no batch-to-batch variation. Nano-Traps are Nanobodies conjugated to beads and are ready-to-use. They enable IP performance superior to that of conventional IgG antibodies.

Alpaca | Conventional antibody | Alpaca heavy chain antibody | Nanobody/ V_HH | Nano-Trap: Nanobody conjugated to bead

ChromoTek Nano-Traps

In addition to the GFP-Trap®, ChromoTek offers Nano-Traps for IP of the below proteins and peptide tags:

Fluorescent protein tags	Peptide tags	Solubilization tags	Oncology
GFP-Trap®	DYKDDDDK Fab-Trap®*	MBP-Trap	Mdm4/HdmX-Trap
mNeonGreen-Trap	Myc-Trap®	GST-Trap	MK2-Trap
RFP-Trap®	Spot-Trap®		p53-N-term-Trap
TurboGFP-Trap	V5-Trap®		p53-C-term-Trap
Halo-Trap			PARP1-Trap
SNAP/CLIP-tag-Trap			

*Note that the DYKDDDDK Fab-Trap® contains a Fab-Fragment

Specificity

The GFP-, mNeonGreen-, TurboGFP-, and RFP-Trap® bind to the following derivatives of fluorescent proteins

Fluorescent protein tags	Peptide tags
GFP-Trap®	AcGFP, Clover, eGFP, Emerald, GFP, GFP5, GFP Envy, GFP S65T, mGFP, mPhluorin, PA-GFP, Superfolder GFP, TagGFP, TagGFP2 CFP YFP, Citrine, eCitrine, eYFP, Venus, Ypet BFP
mNeonGreen-Trap	mNeonGreen
TurboGFP-Trap	TurboGFP, maxGFP
RFP-Trap®	mCherry, mKate2, mOrange, mPlum, mRFP, mRFPruby, mScarlet, mScarlet-I, PA-mCherry, TagRFP

For a complete list see **ptglab.com**

ON THE COVER: *Immunoprecipitation of GFP-fusion proteins*

GFP-TRAP®

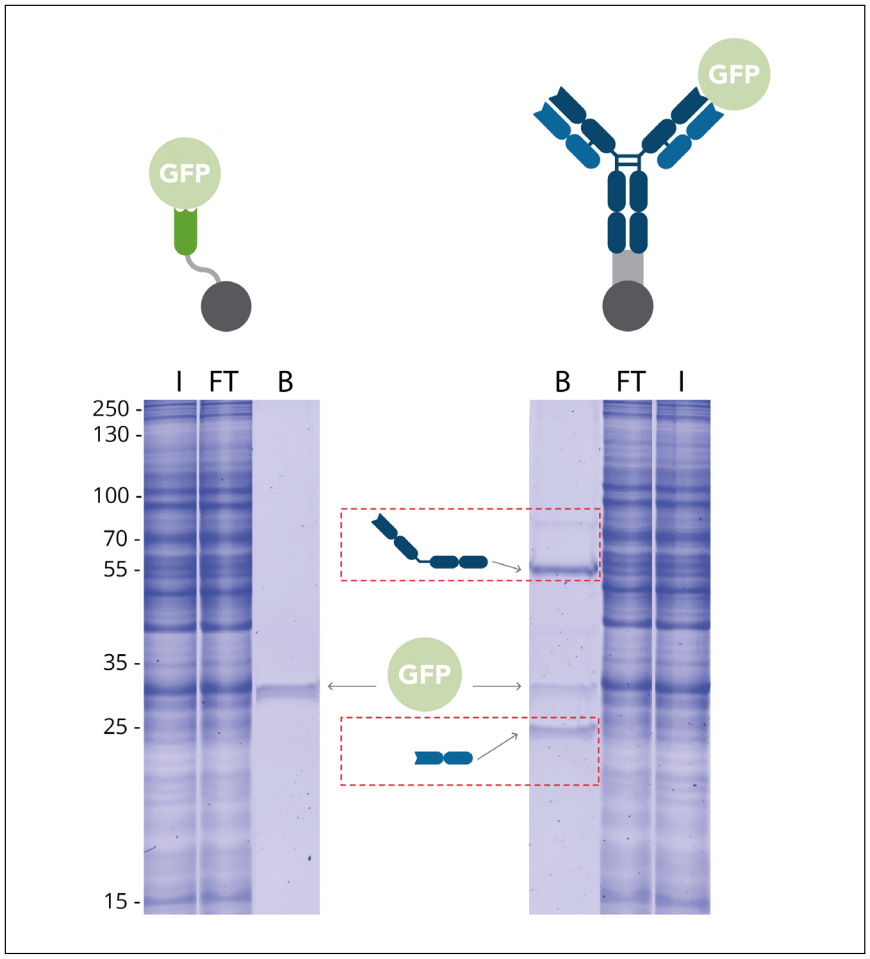
Ready-to-use beads for fast and efficient immunoprecipitation

The GFP-Trap® belongs to ChromoTek's Nano-Trapfamily of ready-to-use pull-down reagents. GFP-Trap® is the benchmarking reagent for one-step immunoprecipitation (IP) of GFP-fusion proteins. The GFP-Trap® consists of an anti-GFP Nanobody coupled to beads. The ChromoTek GFP-Trap® provides a higher level of immunoprecipitation performance than conventional anti-GFP antibodies. Next to immunoprecipitation, GFP-Trap® can be applied in Co-IP, Co-IP/-mass spectrometry, on-bead assays, and ChIP/RIP analysis.

No heavy and light chain antibody fragments & superior background

Benefits of GFP-Trap®:

- No heavy & light antibody chains
- More effective immunoprecipitation
- Less background

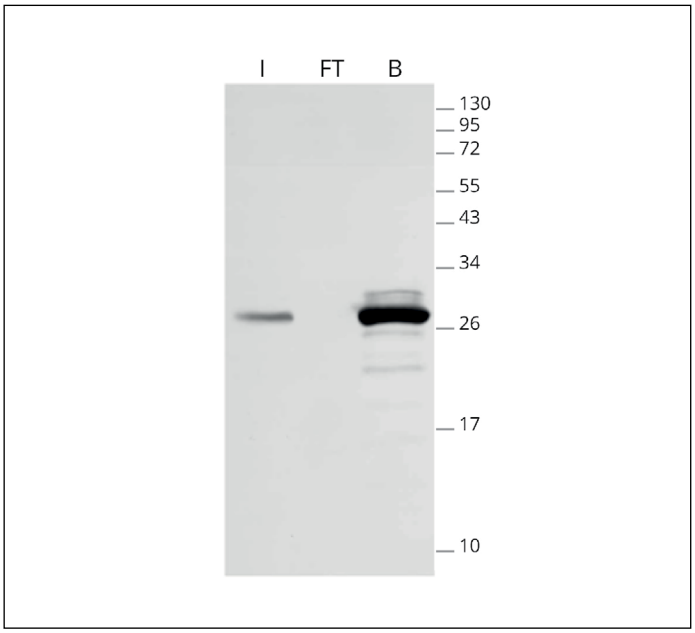


▲ Immunoprecipitation of GFP by GFP-Trap® compared with a conventional anti-GFP antibody coupled to Protein A/G beads analyzed by SDS-PAGE. I: Input, FT: Flow-Through, B: Bound.

When using GFP-Trap® for pull-down of GFP-fusion proteins, the amount of immunoprecipitated GFP is significantly higher and the background is reduced, in contrast to an IP conducted with conventional anti-GFP antibody conjugated to Protein A/G beads. Here, the heavy and light antibody chains (dashed, red boxes) are contaminating. GFP-Trap®, however, provides pure fractions of immunoprecipitated GFP-fusion protein without contamination of heavy & light antibody chains.

Complete pull-down of GFP-tagged proteins with GFP-Trap®

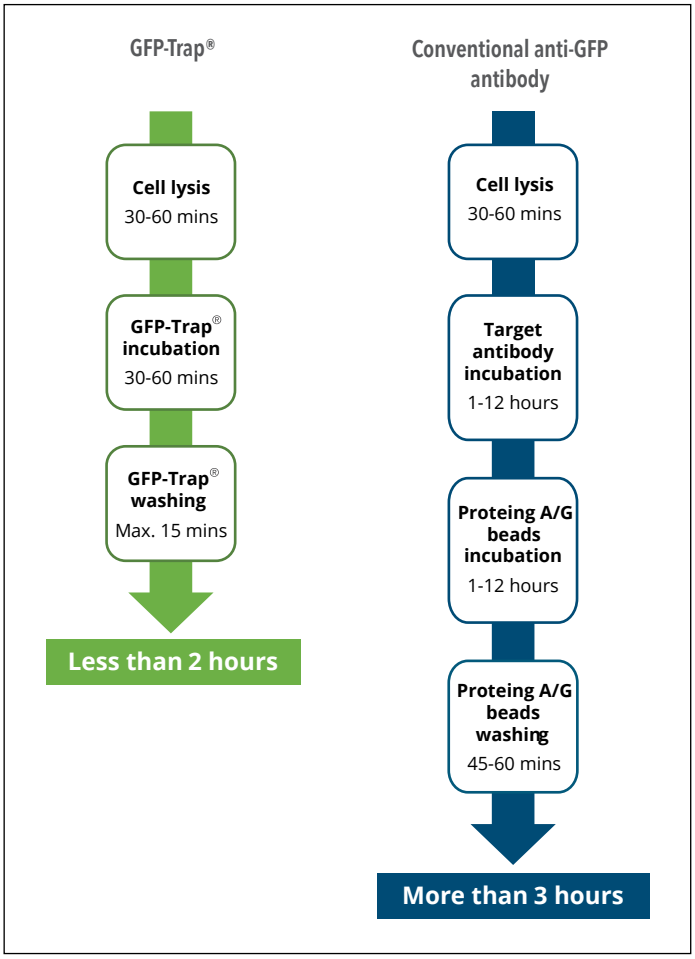
GFP-Trap® effectively immunoprecipitates GFP-fusion proteins. Because of GFP-Trap®'s high binding affinity with a dissociation constant K_D = 1 pM, all GFP-fusion protein is enriched in the bound fraction and no GFP-fusion protein is detectable in the flow-through fraction by Western blotting, indicating a complete pull-down.



▶ Effectiveness of GFP immunoprecipitation with GFP-Trap®: No GFP-fusion protein is detectable in the flow-through fraction by Western blotting, indicating that the GFP-IP was complete because of GFP-Trap®'s high binding affinity K_D = 1 pM. I: Input, FT: Flow-Through, B: Bound.

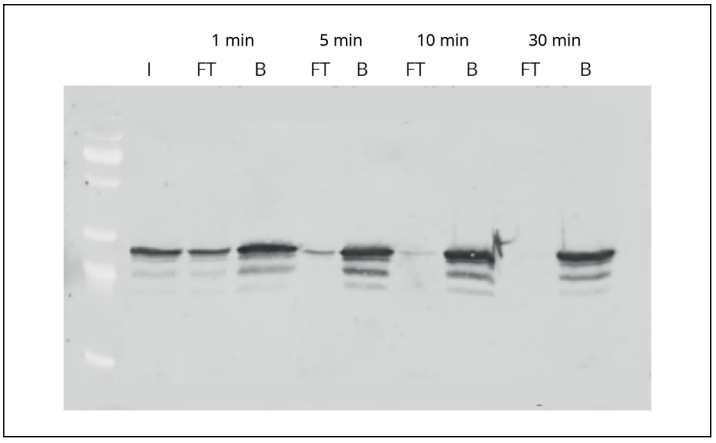
Fast immunoprecipitation

The GFP-Trap® is a ready-to-use reagent that consists of an anti-GFP Nanobody conjugated to beads. Hence, it does not need the additional incubation step required when using Protein A/G beads and conventional antibodies for immunoprecipitation. Experimental and hands-on times are significantly reduced.



▶ Comparison of the duration of immunoprecipitation using GFP-Trap® reagents vs. a conventional anti-GFP antibody, saving significant experimental time of more than 1 hr.

The time course shows the efficient and fast binding of GFP to GFP-Trap®. The high binding rate of the ChromoTek GFP-Trap® enables a considerably shorter processing time than conventional anti-GFP antibodies. For GFP-Trap®, a 30-60 min incubation time at 4°C is sufficient for a complete immunoprecipitation of GFP. Longer incubation times may increase the background of nonspecifically bound proteins instead.



▲ Western Blot of GFP-IP using GFP-Trap®. Input (I) plus flow-through (FT) and bound (B) fractions shown after incubation times of 1, 5, 10, and 30 minutes. The disappearance of the FT lane indicates that the pull-down of GFP is completed after 30 minutes.

Most cited monoclonal anti-GFP antibody

The GFP-Trap® is the most frequently cited monoclonal anti-GFP antibody and the gold standard for immunoprecipitation of GFP-fusion proteins.

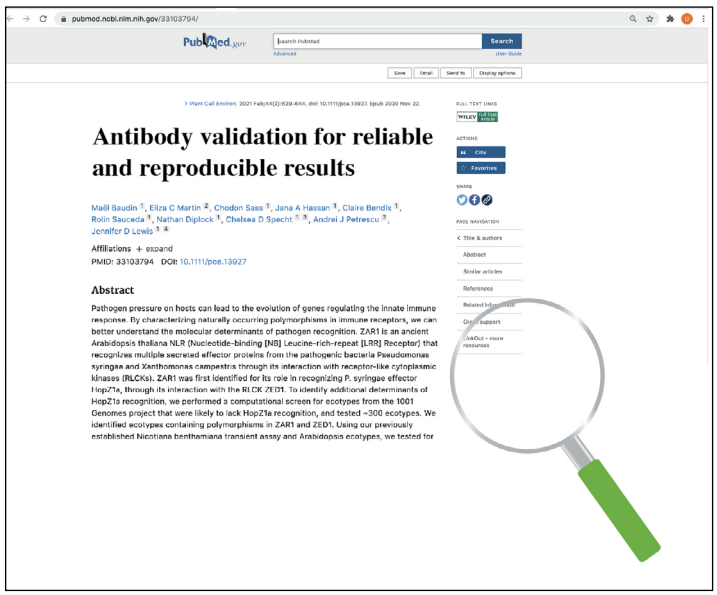
Find references, application notes, and white papers on ptglab.com.

goldstandard
for IP of GFP-fusion proteins

Validation and Characterization

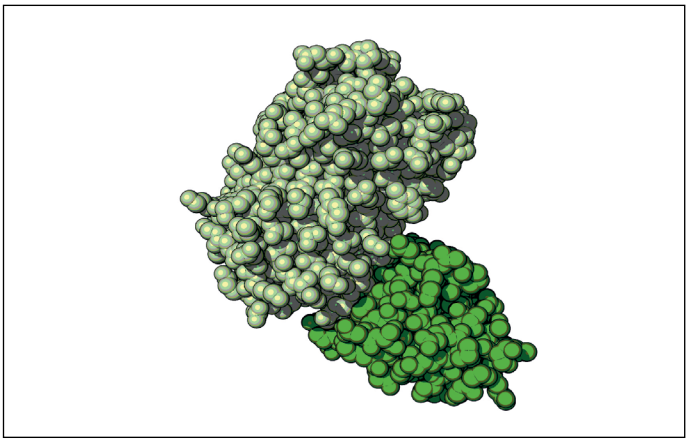
For robust and reproducible experiments, a thorough characterization of antibodies and Nanobodies is extraordinarily important. A comprehensive set of guidelines for the validation of antibodies was published in "A proposal for validation of antibodies" by the International Working Group for Antibody Validation (M. Uhlen et al. 2016). Based on this paper, ChromoTek Nanobodies are validated as follows:

- In the genetic approach, Nano-Traps are tested in their target application immunoprecipitation both on cell lines that express and do not express their cognate fluorescent protein or peptide tag.
- In addition, ChromoTek's Nanobodies are bench marked with established conventional antibodies.



Reliability

Both the sequence and structure of the GFP Nanobody used in the GFP-Trap® are known. The recombinant production in combination with high QC standards ensures reliable and stable alpaca single domain antibody products with virtually no lot-to-lot-variations.

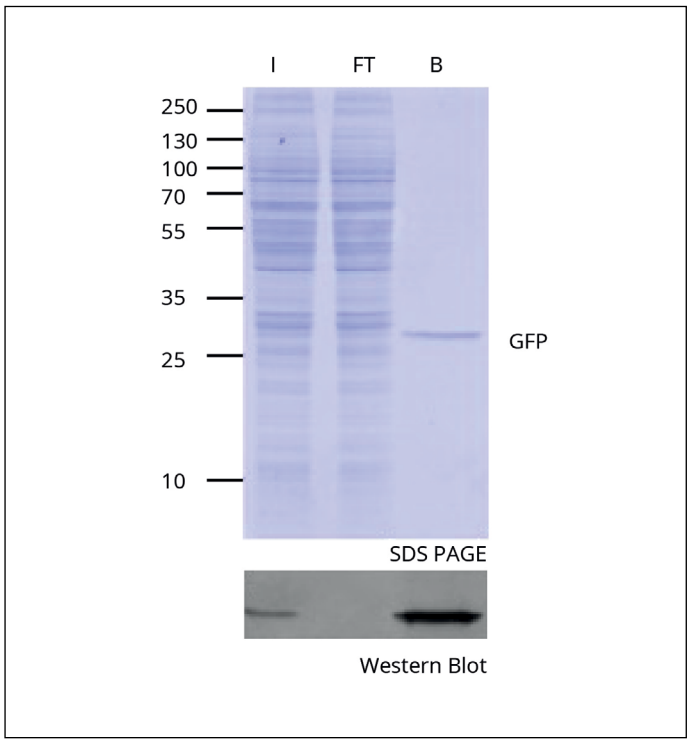


▲ Structure of GFP-GFP Nanobody complex. GFP (light green), GFP Nanobody (dark green).

GFP-Trap® for a higher level of performance in various applications

The GFP-Trap® can be used for immunoprecipitation, including:

- IP of proteins expressed at low levels
- IP of proteins from large volumes, for example, secreted fusion proteins
- IP of membrane proteins in buffers containing detergents
- Co-IP, including Co-IP/MS with high reproducibility and low background
- On-bead enzymatic assays and on-bead digestion for mass spectrometry analysis
- Chromatin/RNA Immunoprecipitation (ChIP, RIP)

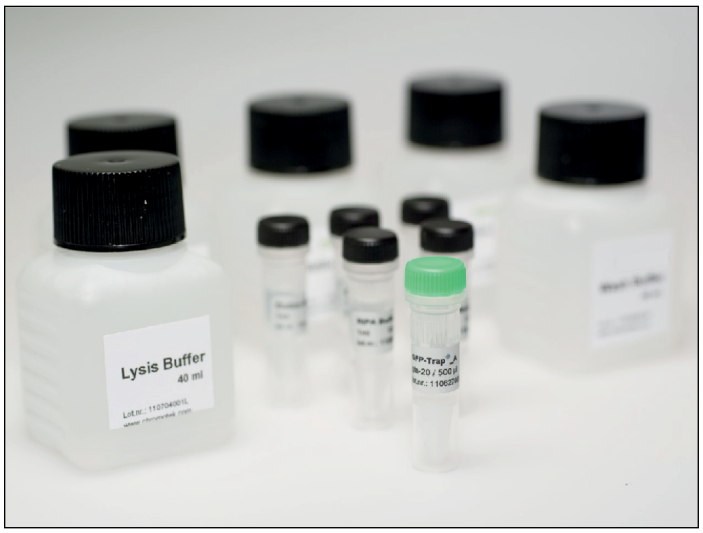


▲ Immunoprecipitation of GFP-fusion proteins: Just the protein of interest - no antibody contamination. Note the effectiveness of pull-down: no GFP detectable in Western blot of the FT fraction, showing complete IP of GFP. I: Input, FT: Flow-Through, B: Bound.

Ready-to-use GFP-Trap® formats

The GFP-Trap® consists of an anti-GFP Nanobody conjugated to different resins. The GFP-Trap® is available as Agarose, Magnetic Agarose, Magnetic Particles M-270, or 96-well plates.

- GFP-Trap® Agarose for the lowest background and high binding capacity IP
- GFP-Trap® Magnetic Agarose for magnetic separation and high binding capacity IP
- GFP-Trap® Magnetic Particles M-270 for pull-down of large proteins/complexes
- GFP-Trap® Multiwell Plates for high-throughput applications and ELISA
- GFP-Trap® kits that include lysis buffer for mammalian cells, wash, and elution buffers

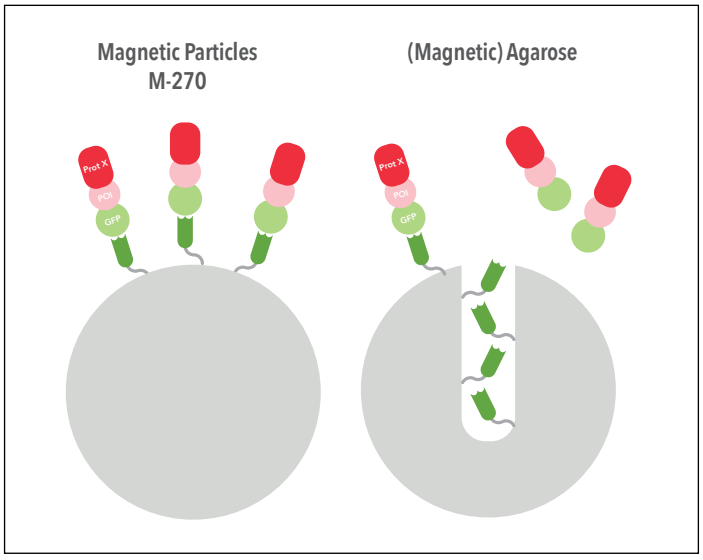


▲ GFP-Trap® Agarose kit

Immunoprecipitation of large proteins/protein complexes

GFP-Trap® Magnetic Particles M-270 are recommended for the investigation of large proteins, protein complexes, or multimers (> 200 kDa). Magnetic Particles M-270 are solid beads, whereas Magnetic Agarose and Agarose are porous beads; large GFP fusion proteins, multimers, or bulky complexes with binding partners may be too large to diffuse into the pores of those agarose-based beads and, therefore,- cannot be bound effectively.

Please note, 200 kDa is an approximate value based on empirical studies. The cut-off can also depend on the protein shape.

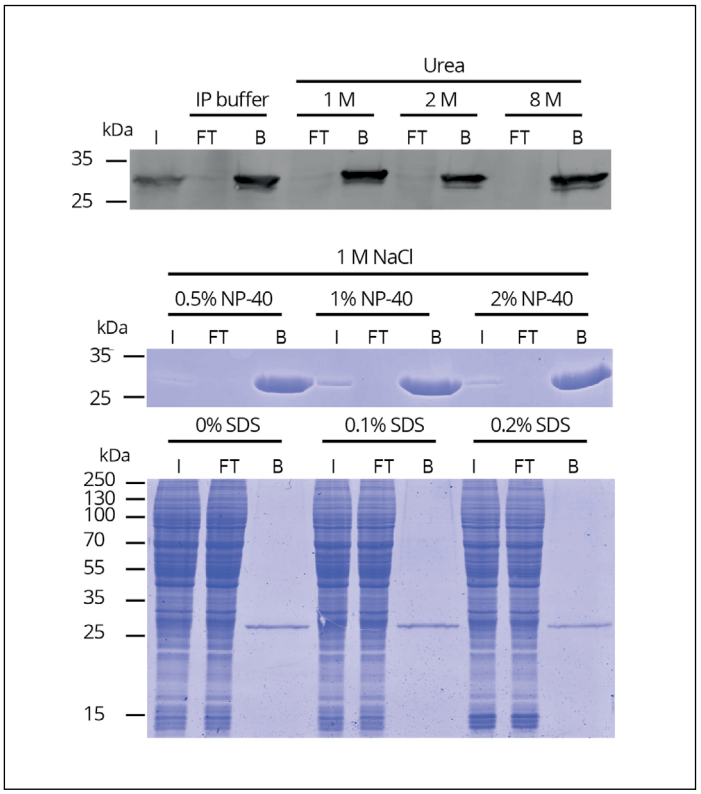


▲ Visualization of the binding of large GFP-fusion proteins (GFP, light green) + protein of interest (POI, pink) with an interacting partner (Prot X, red) to the GFP Nanobody (dark green) of GFP-Trap® Magnetic Particles M-270 (left) and GFP-Trap® (Magnetic) Agarose (right).

Stringent washing

The GFP-Trap® is highly stable in contrast to conventional antibodies. Once bound to the GFP-fusion protein, very stringent washing conditions can be applied to remove unwanted proteins and reduce background. This stability also ensures that the GFP-Trap® can be used in virtually any lysis buffer, e.g., in ubiquitination assays or in the presence of Urea, which is used for the total inactivation of any phosphatase activity in Co-IP/MS for phosphorylation studies.

As you can see in the figure (right), GFP is always effectively bound, no GFP can be detected in flow-through as shown in the Western blot for up to 8 M Urea (left, top) and in the SDS-PAGE for up to 2% NP-40 and 1 M NaCl (left, middle).



▶ Analysis of buffer compatibility: The GFP-Trap® is compatible with common wash buffers and lysis buffer and is also stable under harsh conditions. Even buffers containing 1 M NaCl and 2% NP-40, 1 M NaCl and 0.2% SDS, or 8 M Urea can be used for stringent conditions without compromising GFP-Trap®'s performance. Note that GFP is always effectively bound, no GFP can be detected in flow-through as shown in the Western blot for up to 8 M Urea (top) and in the SDS-PAGE for up to 2% NP-40 and 1 M NaCl (middle).

Wash buffer compatibility of GFP-Trap®

The wash buffer compatibility of the GFP-Trap® bound to GFP has been tested under various conditions. Even denaturing conditions such as 8 M Urea have been shown not to interfere with the binding of GFP. We tested the binding of GFP-Trap® to GFP under reducing conditions, with chaotropic reagents, different salt concentrations, non-ionic polyols, and under higher temperatures. The complex showed unique thermal and chemical stability.

Reducing conditions	1 mM DTT*
	0.2 mM TCEP
Chaotropic reagents	3 M Guanidinium•HCl
	8 M Urea
Salts	2 M NaCl
Detergents	2% Nonidet P40 Substitute
	1% SDS*
	1% Triton X-100
	3% Deoxycholate
Non-ionic polyols	30% Glycerol
Temperature	up to 68°C

▲ GFP-Trap® wash buffer compatibility. *GFP-Trap® Magnetic Particles M-270: 10 mM DTT; 0.2% SDS

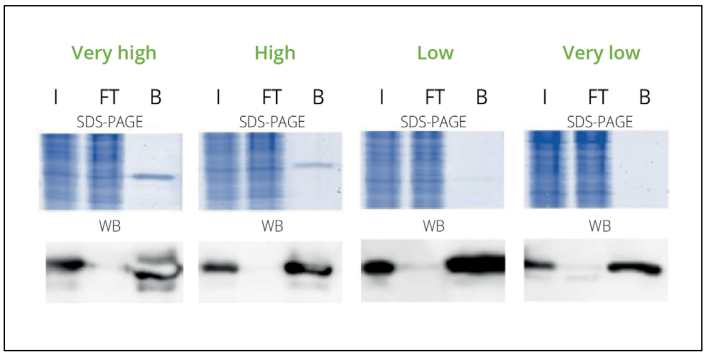
Immunoprecipitation of fusion proteins expressed at low levels

The success of an IP depends on the expression level, specifically the concentration of the protein of interest (POI) in the sample: When the POI concentration equals the value of the dissociation constant of the affinity resin, 50% of the POI is bound. Hence, a high affinity, i.e., low dissociation constant affinity resin is required for the effective IP of low expressed/low abundance POIs, POIs in large volumes such as cell supernatants, etc.

Because the concentration of POIs in the sample/lysis buffer is generally not known to researchers, we used GFP-Trap® to immunoprecipitate EGFP samples at four different concentrations that are typical for very high, high, low, and very low expression/abundance levels when dissolved in standard lysis buffer volumes.

The effectiveness of the EGFP pull-down was analyzed by SDS-PAGE and Western blotting, indicating that the GFP-Trap® was able to effectively immunoprecipitate EGFP at all concentration levels tested, including the very low abundance level because of the GFP-Trap®'s extraordinarily high affinity with a dissociation constant K_D of just 1 pM. In addition, no EGFP was detected in the flow-through fraction even when very high EGFP concentrations were applied, showing the broad applicability of the GFP-Trap® .

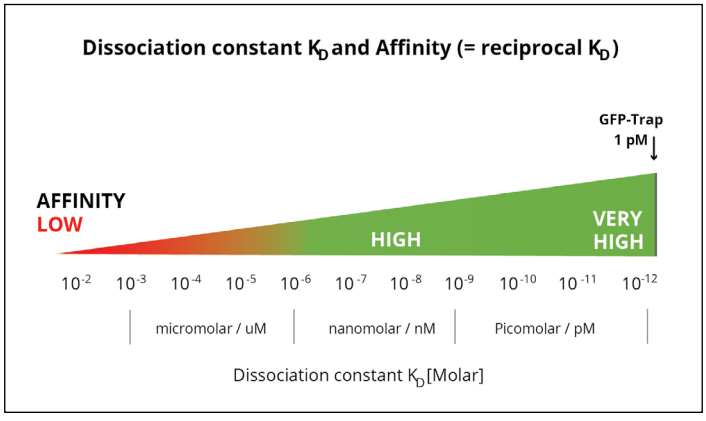
The GFP-Trap® has an extraordinarily high affinity with a dissociation constant K_D of just 1 pM and hence can effectively pull down low abundant GFP-fusion protein, which is present at low concentrations in the sample.



▲ Immunoprecipitation of various concentrations of enhanced GFP (EGFP) with GFP-Trap® Agarose: EGFP in a range from very high to very low amounts is effectively immunoprecipitated by the GFP-Trap®.

Very high corresponds to a concentration of 372 nM and an amount of 5 µg EGFP, high to a concentration of 46 nM and an amount of 0.63 µg EGFP, low to a concentration of 5 nM and an amount of 0.08 µg EGFP, and very low to a concentration of 0.7 nM and an amount of 0.01 µg EGFP in lysis buffer.

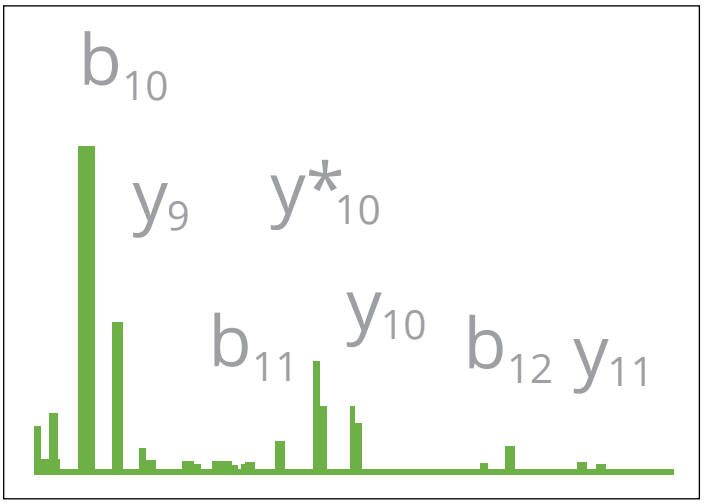
The bound fraction in the Western blot shows a strong signal in all four concentrations whereas the flow-through is almost completely depleted. Especially for proteins expressed at very low levels, the strong band in the bound lane (WB) demonstrates the excellent properties of the GFP-Trap®. Note that a shorter exposure time was used for the WB analysis of very high and high GFP concentrations. I: Input, FT: Flow-Through, B: Bound.



▲ Classification of affinities from low to very high and their translation into dissociation constants. Note, GFP-Trap has a very high affinity with a K of 10 M or 1 pM. Most conventional antibodies typically have dissociation constants from mid-nanomolar (10 M) to micromolar (10 M). ChromoTek's Nano-Traps have higher affinities, i.e. a lower K , which are in the single-digit nanomolar (10 M) to low picomolar (10 M) range and are highly suitable for IP of low abundance proteins.

Co-IP for Mass Spectrometry analysis

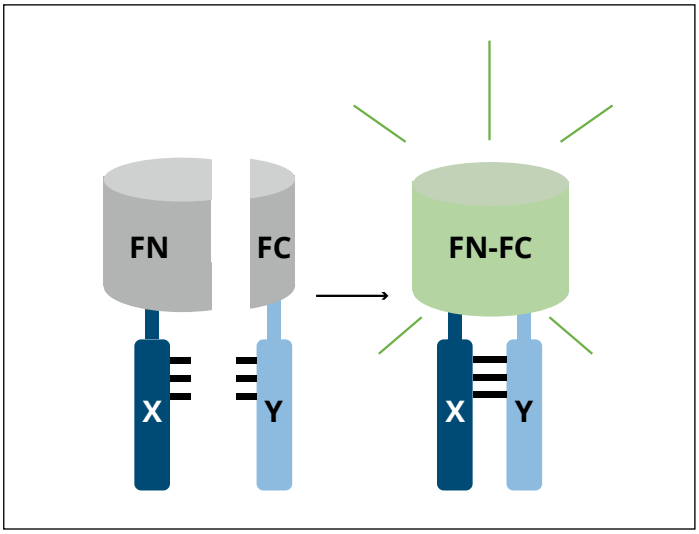
The GFP-Trap® is frequently used for Co-IP/mass spectrometry (MS) assays because of its high reproducibility and low background, which are important for consistent results. Even in the presence of Urea, which can be used for instant inhibition of phosphorylation in the sample, the GFP-Trap® maintains its high specificity and selectivity. After Co-IP, MS sample preparation does not require elution of the bound protein from the beads; instead, it can be conducted on bead either by following ChromoTek's "on-bead digest protocol for mass spectrometry" or using the iST Kit for IP/Co-IP of GFP-fusion proteins & sample preparation MS. This convenient kit comprises the GFP-Trap® and PreOmics iST buffers and cartridges required for easy, convenient, and effective proteomic sample preparation.



▲ Section of a mass spectrum

Split Fluorescent Protein Assays

Split fluorescent protein (FP) assays are used for the analysis of protein-protein interactions. These assays are based on fragments of FPs that are fused to interacting proteins. Once the interacting proteins bind to each other, the fragments of the FPs are also brought into close proximity and, therefore, can reconstitute an active FP. The re-assembling of an FP from its fragments is also known as protein complementation. Here, the GFP-Trap®, mNeonGreen-Trap, and RFP-Trap® are attractive research tools for the biochemical validation of such experiments because they bind reconstituted FPs. For example, GFP-Trap® does not bind the common split fragments GFP1-7 and GFP8-11 but the whole, reconstituted GFP protein.

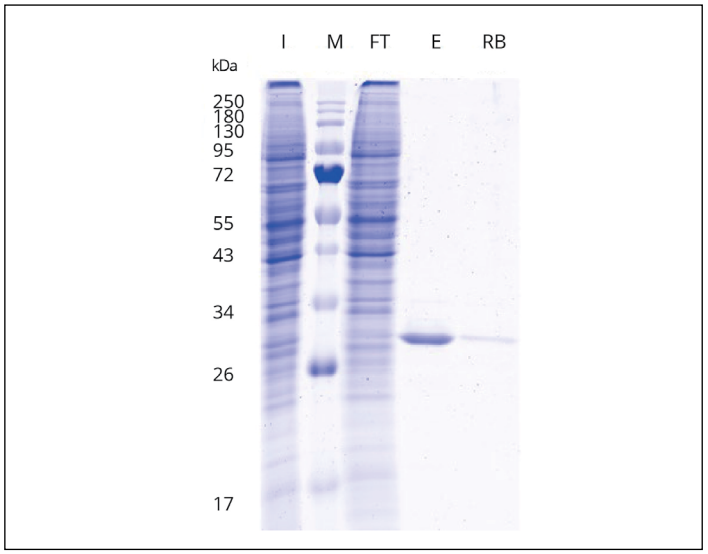


▲ Split fluorescent protein self-complementation assay: The two FP fragments FN and FC are fused to potentially interacting proteins X and Y. It is only upon the binding of their interacting fusion partners that the FP fragments re-assemble and form the active, i.e. fluorescent, complements FN-FC. This is a mechanism of conditional complementarity called "bimolecular fluorescence complementation" (BiFC), first described by Hu et al., 2002. FP can be GFP, mNeonGreen, and RFP.

Elution of Myc-tagged and V5-tagged proteins

In general, fusion proteins can be eluted from Nano-Traps by acidic elution with glycine (pH2.5). It may also be necessary to pipet beads up and down for elution. In addition, Myc- or V5-tagged proteins bound to Myc-Trap® or V5-Trap® can be effectively eluted by using either 2xMyc-peptide or V5-peptide respectively:

- Incubation of 100 µL of diluted 2xMyc-peptide (0.1 mg/mL in PBS) for 10-20 min
- Incubation of 100 µL of diluted V5-peptide (0.72 mg/mL in PBS) for 10-20 min

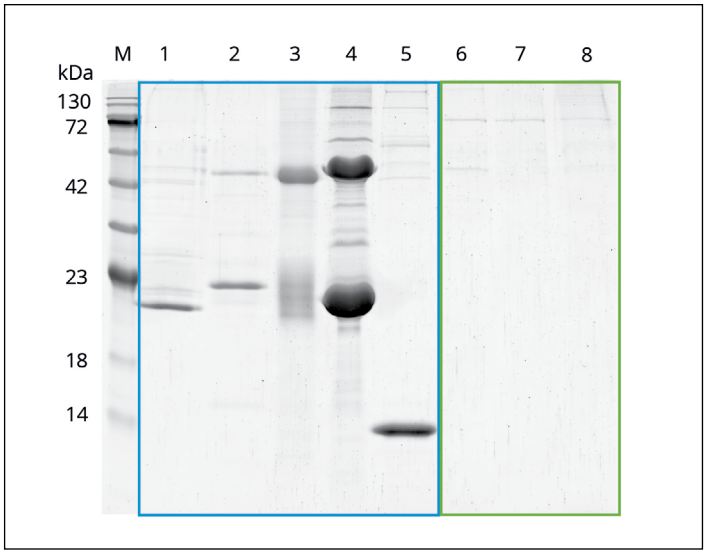


▲ Elution of V5-tagged protein from V5-Trap® Agarose: Competitive elution of a bound V5-tagged protein was performed by 1 h incubation with 100 µL V5-peptide (500 µM in PBS) at 4 °C. Note effective elution at 4 °C with only small amounts of V5-tagged protein left in the residual bound fraction. I: Input, M: Marker, FT: Flow-Through, E: Elution, RB: Residual bound.

Superior background in immunoprecipitation

A comparison of the background of various immunoprecipitations using a variety of commonly used affinity reagents demonstrates that the background of the ChromoTek Spot-Trap® is the lowest. In fact, Spot-Trap® has the lowest, i.e., best, background in class.

The Spot-Trap® is part of ChromoTek's proprietary Spot-System, the first peptide tag-specific Nanobody for universal capture & detection applications. Spot-Trap®, an anti-Spot-tag® Nanobody conjugated to beads, has been developed for the effective immunoprecipitation of Spot-tagged proteins. The Spot-tag® is a short, inert 12 amino acid peptide (PDRVRAVSHWSS). For details see our website.



▲ Comparison of the background of various IP affinity media: IPs were conducted from HEK293T cell lysates with no tagged proteins present, normalized for equal binding capacity, and conducted according to the respective manufacturer's protocol. M: Marker, 1: GST beads, 2: anti-Myc 9E10 antibody beads (supplier A), 3: anti-Myc 9E10 antibody beads (supplier B), 4: anti-Flag beads, 5: Streptavidin conjugated Dynabeads, 6: Spot-Trap® Agarose, 7: Spot-Trap® Magnetic Agarose, 8: Spot-Trap® Magnetic Particles M-270. Lanes 1-5 (blue box) show the unspecific binding of cellular proteins. In contrast, the Spot-Traps® (lanes 6-8, green box) have significantly less background. This makes Spot-Trap® a superior affinity resin for single-band immunoprecipitation.

proteintech®

CONTACT US

Proteintech Germany GmbH
ChromoTek GmbH

Am Klopferspitz 19
82152 Planegg-Martinsried
Germany

P +49 89 124 148 850
F +49 89 124 148 811
E germany@ptglab.com

Proteintech Europe

4th Floor, 196 Deansgate
Manchester
M3 3WF. United Kingdom

P +44 161 839 3007
F +44 161 241 3103
E europe@ptglab.com

Proteintech Group, Inc.
(HQ in North America)

5500 Pearl Street, Ste 400
Rosemont, IL 60018, USA

P +1 (888) 478-4522 (toll free in USA)
F +1 (773) 598-4321
+1 (847) 928-2189
E proteintech@ptglab.com

ptglab.com