GFP-Trap® Multiwell Plate

Product code: gtp-96

Introduction

The ChromoTek GFP-Trap® Multiwell Plate consists of an anti-Green Fluorescent Protein (GFP) Nanobody (VHH), which is immobilized on a transparent 96 multiwell plate via biotin-streptavidin. GFP-Trap Multiwell Plate is used to immunoprecipitate GFP-fusion proteins from cell extracts of various organisms like mammals, plants, bacteria, yeast, insects etc. GFP-Trap Multiwell Plate can be applied in colorimetric, chemiluminescent and fluorescent detection methods.

Properties

Ligand: Anti-GFP single domain antibody fragment (VHH, Nanobody)

Reactivity: Specifically binds to most common GFP derivatives (visit www.chromotek.com for a complete list of recognized GFP variants).

Binding capacity: 200-500 ng of recombinant GFP per well

Plate type: 96 multiwell plate, polystyrene, transparent, solid F-bottom (flat)

Recommended assay volume per well: 200 µL

Surface coating: biotinylated GFP Nanobody and streptavidin

Plate blocking: BSA and Casein hydrolysate

Buffer compatibility: See *Wash buffer compatibility table*.

Storage conditions: Upon receipt store at +4°C. Do not freeze!

Stability: Stable for 1 year upon receipt.

Shipment: Shipped at ambient temperature.

Suggested buffer compositions

Required buffer solutions for immunoprecipitation

NEW: Update of Wash buffer components.

Buffer	Composition
Lysis buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5 % Nonidet™ P40 Substitute (adjust the pH at +4°C)
RIPA buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.1 % SDS, 1 % Triton™ X-100, 1 % deoxycholate (adjust the pH at +4°C)
Dilution buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA (adjust the pH at +4°C)
Wash buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.05 % Nonidet™ P40 Substitute, 0.5 mM EDTA (adjust the pH at +4°C)
2x SDS-sample buffer	120 mM Tris/Cl pH 6.8, 20 % glycerol, 4 % SDS, 0.04 % bromophenol blue, 10 % β-mercaptoethanol
Acidic elution buffer	200 mM glycine pH 2.5 (adjust the pH at +4°C)
Neutralization buffer	1 M Tris pH 10.4 (adjust the pH at +4°C)

Note: Use your equivalent cell lysis buffer for other cell types like yeast, plants, insects, bacteria.

Note: Consider using a Wash buffer without detergent for co-immunoprecipitation.

Required buffer solutions for ELISA

Buffer	Composition
Lysis buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5 % Nonidet™ P40 Substitute (adjust the pH at +4°C)
RIPA buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.1 % SDS, 1 % Triton™ X-100, 1 % deoxycholate (adjust the pH at +4°C)
Dilution	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA (adjust the pH at +4°C)
Phosphate buffered saline (PBS)	10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl
Blocking buffer	PBS, 5% skimmed milk
ELISA Wash buffer	PBS, 0.05% Tween 20
Sulfuric acid	2 M sulfuric acid

Wash buffer compatibility table

Buffer ingredients	Max. concentration
β-mercaptoethanol	10 mM
DTT	10 mM
NaCl	2 M
Nonidet™ P40 Substitute	tested up to 2 %
SDS	0.2 %
TCEP	10 mM
Triton™ X-100	tested up to 2 %
Urea	4 M

Product sizes

Product	Product code	Size
GFP-Trap® Multiwell Plate	gtp-96	96 wells / 96 reactions

Immunoprecipitation protocol

Cell material

The following protocol describes the preparation of mammalian cell lysate!
For other type of cells, we recommend using 500 µg of cell extract and start the protocol with step *Protein Binding*.

Mammalian cell lysis

Note: Harvesting of cells and cell lysis should be performed with ice-cold buffers. We strongly recommend to add protease inhibitors to the Lysis buffer to prevent degradation of your target protein and its binding partners.
For one immunoprecipitation reaction, we recommend using ~10^6-10^7 cells (sufficient for ~3 - 6 wells).

1. Choice of lysis buffer:
   - For cytoplasmic proteins, resuspend the cell pellet in 200 µL ice-cold Lysis buffer by pipetting up and down. Supplement Lysis buffer with protease inhibitor cocktail and 1 mM PMSF (not included).
   - For nuclear/chromatin proteins, resuspend cell pellet in 200 µL ice-cold RIPA buffer supplemented with DNaseI (f.c. 75-150 Kunitz U/mL), $MgCl_2$ (f.c. 2.5 mM), protease inhibitor cocktail and PMSF (f.c. 1 mM) (not included).
2. Place the tube on ice for 30 min and extensively pipette the suspension every 10 min.
3. Centrifuge cell lysate at 17,000x g for 10 min at +4°C. Transfer cleared lysate (supernatant) to a pre-cooled tube and add 400 µL Dilution buffer supplemented with 1 mM PMSF and protease inhibitor cocktail (not included). If required, save 50 µL of diluted lysate for further analysis (input fraction).

Protein binding

1. Add 100-200 µL diluted lysate to each well.
2. Incubate for 1 hour at +4°C.

Note: Fill each well with 200 µL diluted lysate for maximum binding capacity. 600 µL diluted lysate are sufficient for 3-6 wells.
Note: The amount of cells and buffer volumes depend on the expression level of the GFP-fusion protein and on the type of cells. Optimal volumes and concentrations should be determined by the user.
Optional: It is recommend to seal wells that are not used with tape to avoid cross-contamination. Partially used plates should be sealed properly, stored at +4°C and used up as soon as possible.

Washing

1. If required, save 50 µL of supernatant for further analysis (flow-through/non-bound fraction).
2. Remove the supernatant.
3. Wash each well with 300 µL Wash buffer.
4. Remove the supernatant.
5. Repeat this step at least twice.

Optional: To increase stringency of the Wash buffer, test various salt concentrations e.g. 150-500 mM, and/or add a non-ionic detergent e.g. Triton™ X-100 (see *Wash buffer compatibility table* for maximal concentrations).

Elution with 2x SDS-sample buffer (Laemmli)

1. Remove the remaining supernatant.
2. Heat 2x SDS-sample buffer to +95°C.
3. Add 100-200 µL 2x SDS-sample buffer to each well. Mix well with a pipette.
4. Incubate for 10 min at room temperature. Mix multiple times with a pipette.
5. Transfer the supernatant to a new tube.
6. Analyze the supernatant in SDS-PAGE.

Elution with Glycine-elution buffer

1. Remove the remaining supernatant.
2. Add 200 µL Glycine-elution buffer and constantly pipette up and down for 30-60 sec at +4°C or room temperature.
3. Transfer the supernatant to a new tube.
4. Immediately neutralize the eluate fraction with Neutralization buffer.
5. Repeat this step at least once to increase elution efficiency.

Note: Elution at room temperature is more efficient than elution at +4°C. Prewarm buffers for elution at room temperature.

Sandwich ELISA protocol

Cell material

The following protocol describes the preparation of mammalian cell lysate!
For other type of cells, we recommend using 500 µg of cell extract and start the protocol with *Blocking*.

Mammalian cell lysis

Note: Harvesting of cells and cell lysis should be performed with ice-cold buffers. We strongly recommend to add protease inhibitors to the Lysis buffer to prevent degradation of your target protein and its binding partners.
For one reaction, we recommend using ~10^6-10^7 cells (sufficient for ~3-6 wells).

1. Choice of lysis buffer:
   - For cytoplasmic proteins, resuspend the cell pellet in 200 µL ice-cold Lysis buffer by pipetting up and down. Supplement Lysis buffer with protease inhibitor cocktail and 1 mM PMSF (not included).
   - For nuclear/chromatin proteins, resuspend cell pellet in 200 µL ice-cold RIPA buffer supplemented with DNaseI (f.c. 75-150 Kunitz U/mL), $MgCl_2$ (f.c. 2.5 mM), protease inhibitor cocktail and PMSF (f.c. 1 mM) (not included).
2. Place the tube on ice for 30 min and extensively pipette the suspension every 10 min.
3. Centrifuge cell lysate at 17,000x g for 10 min at +4°C. Transfer cleared lysate (supernatant) to a pre-cooled tube and add 400 µL Dilution buffer supplemented with 1 mM PMSF and protease inhibitor cocktail (not included).

Blocking

1. Block each well with 300 µL Blocking buffer for 1 h at room temperature.
2. Wash each well with 300 µL PBS.
3. Repeat the wash step at least once.

Antigen Capture

1. Add 100-200 µL diluted lysate to each well.
2. Incubate for 1 h at +4°C.

Note: Fill each well with 200 µL diluted lysate for maximum binding capacity. 600 µL diluted lysate are sufficient for 3-6 wells.
Note: The amount of cells and buffer volumes depend on the expression level of the GFP-fusion protein and on the type of cells. Optimal volumes and concentrations should be determined by the user.
Optional: It is recommend to seal wells that are not used with tape to avoid cross-contamination. Partially used plates should be sealed properly, stored at +4°C and used up as soon as possible.

Washing

1. Remove the supernatant.
2. Wash each well with 300 µL ELISA Wash buffer.
3. Remove the supernatant.
4. Repeat this step at least 4 times.

Optional: To increase stringency of the ELISA Wash buffer, test various salt concentrations e.g. 150-500 mM, and/or add a non-ionic detergent e.g. Triton™ X-100 (see *Wash buffer compatibility table* for maximal concentrations).

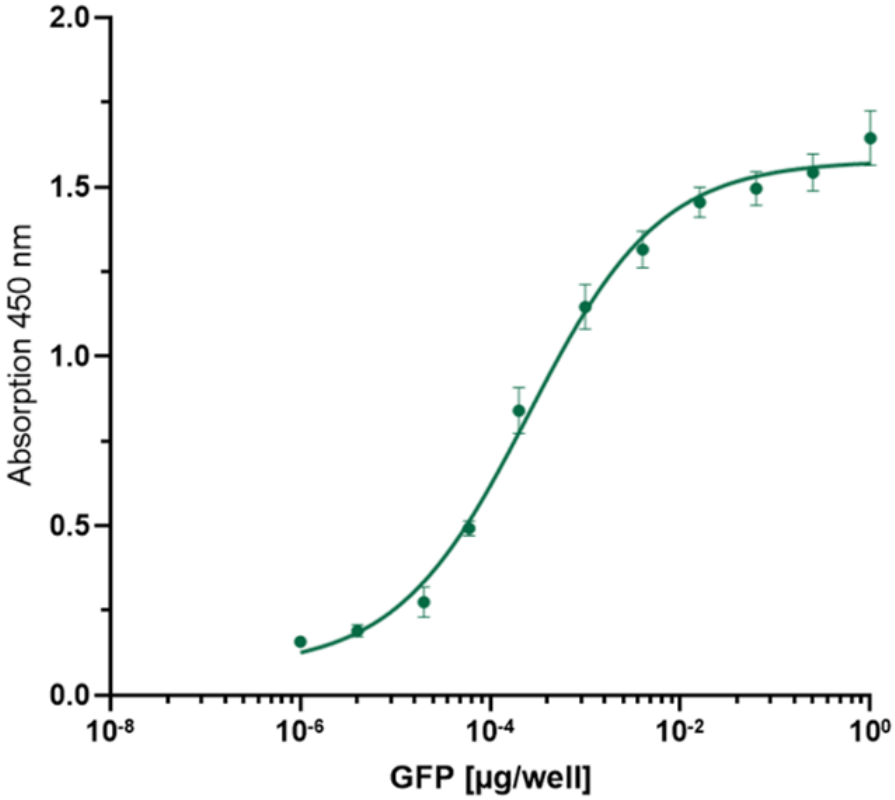
Antibody addition and detection

1. Add 100-200 µL diluted primary antibody to each well. Dilute and incubate the primary antibody as indicated in the manufacturer's manual.
2. Remove the supernatant.
3. Wash each well with 300 µL ELISA Wash buffer.
4. Remove the supernatant. Repeat the wash step at least 4 times.
5. Add 100-200 µL diluted detection antibody to each well. Dilute and incubate the detection antibody as indicated in the manufacturer's manual.
6. Wash each well with 300 µL ELISA Wash buffer.
7. Remove the supernatant. Repeat the wash step at least 4 times.
8. Add 100-200 µL ELISA substrate solution to each well. Incubate the substrate solution as indicated in the manufacturer's manual and stop the reaction with sulfuric acid.
9. Analyze with a photospectrometer.

Note: Anti-His primary antibodies can't be used with GFP-Trap Multiwell Plate.

Optional: Detection of GFP-fusion proteins with anti-GFP antibody [PABG1]

1. Perform *Mammalian Cell Lysis*, *Blocking*, *Antigen Capture*, and *Washing* as described above.
2. Dilute GFP antibody [PABG1] (rabbit polyclonal GFP antibody, ChromoTek GmbH) 1:1,000 in Blocking buffer.
3. Add 200 µL diluted GFP antibody [PABG1] to each well and incubate for 1 h at +4°C.
4. Remove the supernatant and wash each well at least 5 times with 300 µL ELISA Wash buffer.
5. Add 200 µL of the detection antibody to each well (e.g. Anti-rabbit IgG, HRP-linked Antibody, Cell Signalling #7074; dilution 1:1,000 in Blocking buffer; incubation for 1 h at room temperature).
6. Remove the supernatant and wash each well at least 5 times with 300 µL ELISA Wash buffer.
7. Add 100 µL ELISA substrate solution to each well (e.g. 1-Step™ Ultra TMB-ELISA Substrate Solution, ThermoFisher Scientific #34028; incubation for 2-15 min at room temperature).
8. Add 200 µL Sulfuric acid to stop the reaction.
9. Analyze with a photospectrometer.



Standard curve of GFP titrated in HEK 293T cell lysate. Detection with anti-GFP antibody [PABG1] (ChromoTek GmbH), anti-rabbit IgG, HRP-linked Antibody (Cell Signalling #7074) and 1-Step™ Ultra TMB-ELISA Substrate Solution (ThermoFisher Scientific #34028). Absorbance measured at 450 nm.
A minimum of 20 pg GFP per well can be detected.

Product overview and related products

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 Kit	gtdk-20
iST GFP-Trap Kit for IP/MS	gtak-iST-8
GFP-Trap® Multiwell Plate	gtp-96
Binding Control Agarose	bab-20
Binding Control Magnetic Agarose	bmab-20
Spin columns	sct-10; sct-20; sct-50
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
Nano-Secondary® alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor® 488 [CTK0101, CTK0102] Nano-Secondary® alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor® 568 [CTK0101, CTK0102] Nano-Secondary® alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor® 647 [CTK0101, CTK0102]	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	gb2AF488-10; -50 gb2AF568-10; -50 gb2AF647-10; -50 gba488-10; -100 gba594-10; -100 gba647n-10; -100

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

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