DYKDDDDK Fab-Trap™ Agarose

Product code: ffa

Introduction

The ChromoTek DYKDDDDK Fab-Trap™ Agarose consists of an anti-DYKDDDDK-tag Fab fragment, which is covalently bound to agarose beads. DYKDDDDK Fab-Trap™ Agarose is used to immunoprecipitate 1x and 3xDYKDDDDK-tagged fusion proteins from cell extracts of various organisms like mammals, plants, bacteria, yeast, insects etc.

Properties

Ligand: Anti-DYKDDDDK-tag Fab fragment

Clone: Fab fragment of monoclonal mouse IgG1 M2.1

Reactivity: Specifically binds to 1x and 3xDYKDDDDK-tag sequence (1x and 3xFlag®-tag). Compatible with N-, C-terminal or internal tagging.

Binding capacity: 12.5 - 15 µg of recombinant 3xDYKDDDDK-tagged protein (~30 kDa) per 25µL bead slurry

Bead size: 90 µm (cross-linked 4 % agarose beads)

Buffer compatibility: See *Wash buffer compatibility table*.

Storage buffer: 1x PBS, Preservative: 0.09 % sodium azide

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

Stability: Stable for 1 year upon receipt.

Shipment: Shipped at ambient temperature.

RRID: AB_2894836

Suggested buffer compositions

Required buffer solutions

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)
Peptide elution buffer	2 mg/mL 3xDYKDDDDK-peptide (fp-1) reconstituted in PBS

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.

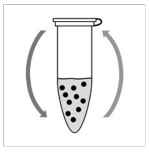
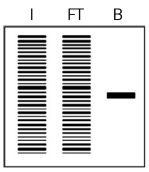
Wash buffer compatibility table

Buffer ingredients	Max. concentration
β-mercaptoethanol	10 mM
CHAPS	0.5 %
DDM	2 %
DTT	10 mM
NaCl	2 M
Nonidet™ P40 Substitute	tested up to 0.5 %
SDS	0.2 %
TCEP	10 mM
Tween™ 20	tested up to 5 %
Urea	2 M

Product sizes

Product	Product code	Size
DYKDDDDK Fab-Trap™ Agarose	ffa-10	10 reactions (250 µL slurry)
	ffa-20	20 reactions (500 µL slurry)
	ffa-100	100 reactions (2.5 mL slurry)
	ffa-200	200 reactions (5 mL slurry)
	ffa-400	400 reactions (10 mL slurry)
DYKDDDDK Fab-Trap™ Agarose Kit	ffak-20	20 reactions (500 µL slurry) including buffers

Protocol at a glance

General		• Perform all steps at +4°. • Use your preferred cell lysis buffer and cell lysis conditions.
Cell Lysis		• Use 10^6-10^7 cells and 200 µL Lysis buffer. • Perform cell lysis and clear lysate. • Mix 200 µL cleared lysate with 300 µL Dilution buffer.
Bead equilibration		• Transfer 25 µL bead slurry into a 1.5 mL tube. • Equilibrate beads 3x with 500 µL Dilution Buffer.
Protein binding		• Add 500 µL diluted lysate to beads. • Rotate end-over-end for 1 hour at +4°C.
Washing		• Wash beads 3x with 500 µL Wash buffer. • Transfer beads to a new tube during the last washing step.
Elution with SDS-sample buffer		• Resuspend beads in 80 µL 2x SDS-sample buffer. • Boil beads for 5 min at +95°C. • Analyze the supernatant in SDS-PAGE / Western Blot.

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 *Bead equilibration*.

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.

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 300 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).

Bead equilibration

1. Resuspend the beads by gently pipetting up and down or by inverting the tube. Do not vortex the beads!
2. Transfer 25 µL of bead slurry into a 1.5 mL reaction tube.
3. Add 500 µL ice-cold Dilution buffer.
4. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard the supernatant.

Note: Alternatively, Spin columns (sct-10; -20; -50) can be used to equilibrate the beads.

Protein binding

1. Add diluted lysate to the equilibrated beads.
2. Rotate end-over-end for 1 hour at +4°C.

Washing

1. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
2. If required, save 50 µL of supernatant for further analysis (flow-through/non-bound fraction).
3. Discard remaining supernatant.
4. Resuspend beads in 500 µL Wash buffer.
5. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard remaining supernatant.
6. Repeat this step at least twice.
7. During the last washing step, transfer the beads to a new tube.

Optional: To increase stringency of the Wash buffer, test various salt concentrations e.g. 150-500 mM, and/or add a non-ionic detergent (see *Wash buffer compatibility table* for maximal concentrations).
Note: Alternatively, Spin columns (sct-10; -20; -50) can be used to wash the beads.

Elution with 2x SDS-sample buffer (Laemmli)

1. Remove the remaining supernatant.
2. Resuspend beads in 80 µL 2x SDS-sample buffer.
3. Boil beads for 5 min at +95°C to dissociate immunocomplexes from beads.
4. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
5. Analyze the supernatant in SDS-PAGE / Western Blot.

Note: For Western blot detection we recommend DYKDDDDK tag Polyclonal antibody (Binds to FLAG® tag epitope) (20543-1-AP).

Elution with Acidic elution buffer

1. Remove the remaining supernatant.
2. Add 50-100 µL Acidic elution buffer and constantly pipette up and down for 30-60 sec at +4°C or room temperature.
3. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
4. Transfer the supernatant to a new tube.
5. Immediately neutralize the eluate fraction with 5-10 µL Neutralization buffer.
6. 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.
Note: Alternatively, Spin columns (sct-10; -20; -50) can be used to separate the beads.

Elution with Peptide elution buffer

1. Reconstitute 3xDYKDDDDK-peptide in PBS to a final concentration of 2 mg/mL (0.7 mM). Vortex for a few minutes to dissolve the powder.
2. Dilute 3xDYKDDDDK-peptide stock to 150 µg/mL (0.05 mM) in PBS.
3. Remove the remaining supernatant from the beads.
4. Add 100 µL of the diluted 3xDYKDDDDK-peptide and mix with a pipette.
5. Mix for 10-20 min at room temperature or for 30-60 min at +4°C.
6. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
7. Transfer the supernatant to a new tube.
8. 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.
Note: Alternatively, Spin columns (sct-10; -20; -50) can be used to separate the beads.

Product overview and related products

DYKDDDDK-tag toolbox	Product code
DYKDDDDK Fab-Trap™ Agarose	ffa-10; -20; -100
DYKDDDDK Fab-Trap™ Agarose Kit	ffak-20
DYKDDDDK Immunoprecipitation Starter Pack	ffap
Binding Control Agarose	bab-20
Spin columns	sct-10; sct-20; sct-50
3xDYKDDDDK-peptide	fp-1
DYKDDDDK tag Polyclonal antibody (Binds to FLAG® tag epitope)	20543-1-AP
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
DYKDDDDK tag Monoclonal antibody (Binds to FLAG® tag epitope)	66008-3-Ig
HRP-conjugated DYKDDDDK Tag Monoclonal antibody (Binds to FLAG® tag epitope)	HRP-66008

For product details, information, and ordering visit www.chromotek.com or www.ptglab.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 Lane
Islandia, NY 11749
USA
phone: 631 501 1058
fax: 631 501 1060

Disclaimer

Only for research applications, not for diagnostic or therapeutic use!