mNeonGreen-Trap Agarose Kit

Product code: ntak-20

Introduction

The ChromoTek mNeonGreen-Trap Agarose Kit consists of an anti-mNeonGreen Nanobody (VHH), which is covalently bound to agarose beads. mNeonGreen-Trap Agarose Kit is used to immunoprecipitate mNeonGreen-fusion proteins from cell extracts of various organisms like mammals, plants, bacteria, yeast, insects etc.

Properties

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

Reactivity: Specifically binds to mNeonGreen from the lancelet *Branchiostoma lanceolatum* (visit www.chromotek.com for a complete list of recognized fluorescent proteins).

Binding capacity: 30 µg of recombinant mNeonGreen per 25 µL bead slurry

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

Buffer compatibility: See *Wash buffer compatibility table*.

Storage buffer: 20 % ethanol

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_2827593

Suggested buffer compositions

Buffers provided in the kit

NEW: Update of Wash buffer components.

Buffer	Composition	Quantity
Lysis buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5 % Nonidet™ P40 Substitute, 0.09 % sodium azide	30 mL
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, 0.09 % sodium azide	30 mL
Dilution buffer*	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.018 % sodium azide	50 mL (after dilution with 40 mL H_2O)
Wash buffer*	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.05 % Nonidet™ P40 Substitute, 0.5 mM EDTA, 0.018 % sodium azide	50 mL (after dilution with 40 mL H_2O)
Acidic elution buffer	200 mM glycine pH 2.5	3x 1 mL

*Add 40 mL H_2O to Dilution buffer and Wash buffer before use. The indicated buffer composition refers to the diluted buffer solution.

Note: Sodium azide is added to buffers as antiseptic and antifungal agent.
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

Buffer	Composition
2x SDS-sample buffer	120 mM Tris/Cl pH 6.8, 20 % glycerol, 4 % SDS, 0.04 % bromophenol blue, 10 % β-mercaptoethanol
Neutralization buffer	1 M Tris pH 10.4 (adjust the pH at +4°C)

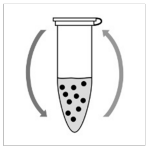
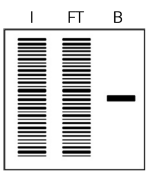
Wash buffer compatibility table

Buffer ingredients	Max. concentration
DTT	10 mM
NaCl	2 M
Nonidet™ P40 Substitute	tested up to 2 %
SDS	0.2 %
Triton™ X-100	tested up to 1 %
Urea	4 M

Product sizes

Product	Product code	Size
mNeonGreen-Trap Agarose	nta-10	10 reactions (250 µL slurry)
	nta-20	20 reactions (500 µL slurry)
	nta-100	100 reactions (2.5 mL slurry)
	nta-200	200 reactions (5 mL slurry)
	nta-400	400 reactions (10 mL slurry)
mNeonGreen-Trap Agarose Kit	ntak-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 e.g. Triton™ X-100 (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 mNeonGreen antibody [32F6] (32f6-20; -100).

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.

Product overview and related products

mNeonGreen toolbox	Product code
mNeonGreen-Trap Agarose	nta-10; -20; -100
mNeonGreen-Trap Agarose Kit	ntak-20
mNeonGreen-Trap Magnetic Agarose	ntma-10; -20; -100
mNeonGreen-Trap Magnetic Agarose Kit	ntmak-20
iST mNeonGreen-Trap Kit for IP/MS	ntak-iST-8
Binding Control Agarose	bab-20
Binding Control Magnetic Agarose	bmab-20
Spin columns	sct-10; sct-20; sct-50
mNeonGreen VHH, recombinant binding protein	nt-250
mNeonGreen antibody [32F6] (mouse monoclonal)	32f6-20; -100

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

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