mNeonGreen-Trap_A for Immunoprecipitation of mNeonGreen-Fusion Proteins from mammalian cell extract

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

Introduction

Fluorescent proteins are widely used to study protein function, localization and dynamics in cells. mNeonGreen is a bright monomeric yellow-green fluorescent protein derived from the lancelet *Branchiostoma lanceolatum*. Lancelet mNeonGreen is evolutionarily distant from jellyfish-derived fluorescent proteins and shares only ~20% sequence identity with the commonly used GFP variants. For biochemical analysis including mass spectrometry and enzyme activity measurements, mNeonGreen-fusion proteins and their interacting factors can be isolated fast and efficiently by immunoprecipitation using the mNeonGreen-Trap. mNeonGreen-Trap_A utilizes small recombinant alpaca antibody fragments covalently coupled to the surface of agarose beads.

Specificity

tested on mNeonGreen

Content

Reagent	Code	Quantity
mNeonGreen-Trap_A	nta-20	20 reactions (0.5 ml slurry)
mNeonGreen-Trap_A	nta-100	100 reactions (2.5 ml slurry)
mNeonGreen-Trap_A	nta-200	200 reactions (5 ml slurry)
mNeonGreen-Trap_A	nta-400	400 reactions (10 ml slurry)

Bead properties

Bead size: ~ 90 µm (cross-linked 4% agarose beads)
Storage buffer: 20% EtOH
Binding capacity: 10 µl mNeonGreen-Trap_A slurry binds 13 µg of recombinant mNeonGreen

Stability and Storage

Shipped at ambient temperature. Upon receipt store at +4°C.
Stable for 1 year. Do not freeze.

Required solutions

Suggested buffer composition

Buffer	Composition
Lysis buffer (CoIP)	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.5% NP-40
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
Dilution/Wash buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Glycine-elution buffer	200 mM glycine pH 2.5
2 x SDS-sample buffer	120 mM Tris/Cl pH 6.8; 20% glycerol; 4% SDS, 0.04% bromophenol blue; 10% β-mercaptoethanol

Related products

mNeonGreen Toolbox	Code
mNeonGreen-Binding protein	nt-250
mNeonGreen-Trap_A Kit	ntak-20
Blocked agarose beads	bab-20
mNeonGreen antibody (WB, IF)	32f6-20; 32f6-100
Spin columns	sct-10; sct-20; sct-50

Support

Please refer to our FAQ section at *www.chromotek.com* or contact *support@chromotek.com*

Protocol for Immunoprecipitation of mNeonGreen-Fusion Proteins using mNeonGreen-Trap_A

Harvest cells

For one immunoprecipitation reaction the use of ~10^6 - 10^7 mammalian cells (approx. one 10-cm dish) expressing a mNeonGreen-tagged protein of interest is recommended. To harvest adherent cells, aspirate growth medium, add 1 ml ice-cold PBS to cells and scrape cells from dish. Transfer cells to a pre-cooled tube, spin at 500 g for 3 min at +4°C and discard supernatant. Wash cell pellet twice with ice-cold PBS, gently resuspending the cells. After washing:

Lyse cells

1. Resuspend cell pellet in 200 µl ice-cold lysis buffer by pipetting or using a syringe.

   note: Supplement lysis buffer with protease inhibitors and 1 mM PMSF (not included).
   optional for nuclear/chromatin proteins: Use RIPA buffer supplemented with 1 mg/ml DNase, 2.5 mM $MgCl_2$, protease inhibitors and 1 mM PMSF (not included).
2. Place the tube on ice for 30 min with extensively pipetting every 10 min.
3. Centrifuge cell lysate at 20.000x g for 10 min at +4°C. Transfer lysate to a pre-cooled tube. Add 300 µl dilution buffer to lysate. Discard pellet.

   note: At this point cell lysate may be put at -80°C for long-term storage.
   optional: Add 1 mM PMSF and protease inhibitors (not included) to dilution buffer.

Equilibrate beads

4. Vortex mNeonGreen-Trap_A beads and pipette 25 µl bead slurry into 500 µl ice-cold dilution buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash twice.

Bind proteins

5. Add diluted lysate (step 3) to equilibrated mNeonGreen-Trap_A beads (step 4). If required, save 50 µl of diluted lysate for immunoblot analysis. Tumble end-over-end for 1 hour at 4°C.
6. Centrifuge at 2.500x g for 2 min at +4°C. If required, save 50 µl supernatant for immunoblot analysis. Discard remaining supernatant.

Wash beads

7. Resuspend mNeonGreen-Trap_A beads in 500 µl ice-cold dilution buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash twice.
   optional: Increase salt concentration in the second washing step up to 500 mM.

Elute proteins

8. Resuspend mNeonGreen-Trap_A beads in 100 µl 2x SDS-sample buffer.
9. Boil resuspended mNeonGreen-Trap® _A beads for 10 min at 95°C to dissociate immunocomplexes from mNeonGreen-Trap_A beads. mNeonGreen-Trap_A beads can be collected by centrifugation at 2.500x g for 2 min at 4°C and SDS-PAGE is performed with the supernatant.
10. *optional instead of steps 8 and 9: elute bound proteins by adding 50 µl 0.2 M glycine pH 2.5 (incubation time: 30 sec under constant mixing) followed by centrifugation. Transfer the supernatant to a new tube and add 5 µl 1M Tris base pH 10.4 for neutralization. To increase elution efficiency this step can be repeated.*