Dnmt1-Trap_A for Immunoprecipitation of Dnmt1

For the immunoprecipitation of endogenous Dnmt1 from cellular extracts.
Only for research applications, not for diagnostic or therapeutic use

1. Introduction

For biochemical analyses including mass spectrometry and enzyme activity measurements endogenous Dnmt1 proteins and their interacting factors can be isolated fast and efficiently in one step by immunoprecipitation using the Dnmt1-Trap. Dnmt1-Trap_A contains a small Dnmt1-binding protein covalently coupled to the surface of agarose beads.

2. Content

Reagent	Code	Quantity
Dnmt1-Trap_A	dta-20	20 reactions (0.5 ml resin)
Dnmt1-Trap_A	dta-100	100 reactions (2.5 ml resin)
Dnmt1-Trap_A	dta-200	200 reactions (5 ml resin)
Dnmt1-Trap_A	dta-400	400 reactions (10 ml resin)

3. Bead properties

Bead size: ~ 90 µm
Storage buffer: 20% EtOH
Binding capacity: 10 µl Dnmt1-Trap_A slurry binds 2.5 – 3 µg of DNMT1

4. Stability and Storage

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

5. Protocol

1. For one immunoprecipitation reaction resuspend cell pellet (~10^7 mammalian cells) in 200 µl lysis buffer by pipetting (or using a syringe).

   optional: add 1 mM PMSF and Protease inhibitor cocktail (not included) to lysis buffer
   optional for nuclear/chromatin proteins: add 1 mg/ml DNase and 2.5 mM $MgCl_2$ (not included) to lysis buffer

2. Place the tube on ice for 30 min with extensively pipetting every 10 min.

3. Spin cell lysate at 20.000x g for 5 -10 minutes at 4°C.

4. Transfer supernatant to a pre-cooled tube. Adjust volume with dilution buffer to 500 µl – 1000 µl. Discard pellet.

   optional: add 1 mM PMSF and Protease inhibitor cocktail (not included) to dilution buffer
   note: the cell lysate can be frozen at this point for long-term storage at -80°C

   For immunoblot analysis dilute 50 µl cell lysate with 50 µl 2x SDS-sample buffer (→ refer to as input).

5. Equilibrate Dnmt1-Trap_A beads in dilution buffer. Resuspend 20 - 30 µl bead slurry in 500 µl ice cold dilution buffer and spin down at 2.500x g for 2 minutes at 4°C. Discard supernatant and wash beads 2 more times with 500 µl ice cold dilution buffer.

6. Add cell lysate to equilibrated Dnmt1-Trap_A beads and incubate the Dnmt1-Trap_A beads with the cell lysate under constant mixing for 10 min – 2 h at room temperature or 4°C.

   note: during incubation of protein sample with the Dnmt1-Trap_A the final concentration of detergents should not exceed 0.2% to avoid unspecific binding to the matrix

7. Spin tube at 2.500x g for 2 minutes at 4°C. For western blot analysis dilute 50 µl supernatant with 50 µl 2x SDS-sample buffer (→ refer to as non-bound). Discard remaining supernatant.
8. Wash beads two times with 500 µl ice cold wash buffer.

   optional: increase salt concentration in the second washing step up to 500 mM
9. Dnmt1-Trap_A beads in 100 µl 2x SDS-Sample buffer or go to step 11.
10. Boil resuspended beads for 10 minutes at 95°C to dissociate the immunocomplexes from the beads. The beads can be collected by centrifugation at 2.500x g for 2 minutes at 4°C and SDS-PAGE is performed with the supernatant (→ refer to as bound).
11. *optional: 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 fresh cup and add 5 µl 1M Tris base (pH 10.4) for neutralization. To increase elution efficiency this step can be repeated.*

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
10x RIPA buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 5 mM EDTA; 0.1% SDS; 1% Triton X-100; 1% Deoxycholate
Dilution buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Wash buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Elution buffer	200 mM glycine pH 2.5

Support/ Troubleshooting

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

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Dnmt1 Chromobody	dcg or dcr

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