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DEEP DV

Nickel-Nitrilotriacetic Acid (NiNTA) Precipitation

For any questions regarding this protocol, feel free to send an email to info@deep-dv.org

The following protocol is specifically designed for the purification of histidine-tagged recombinant proteins. The use of cell lines stably transduced or transiently transfected with His-tagged SUMO or ubiquitin proteins allows to precipitate the entire proteome modified with SUMO or ubiquitin from eukaryotic cells. By using special denaturation buffers, only covalent SUMOylation or ubiquitinylation of target proteins is detected and can thus be distinguished from protein-protein interactions. Subsequent immunoblot blot analysis and/or mass spectrometry can be used to identify the post-translationally modified target proteins.

1. Preparation of Cells

- Use cells stably expressing or transiently transfected with 6xHistidine-tagged SUMO1, SUMO2/3, or ubiquitin.
- Transfect or infect cells according to the experimental setup.
- Harvest cells using a cell scraper in media and transfer them into 15 ml tubes.
- Centrifuge at 2000 rpm for 3 minutes.
- Discard the supernatant and resuspend the cell pellet in 5 ml of 1× PBS.

2. Preparation of Total-Cell Lysates

- Transfer 1 ml of the cell suspension into a fresh 1.5 ml reaction tube.
- Prepare total-cell lysates using RIPA buffer supplemented with the following protease inhibitors:
  - 0.2 mM PMSF
  - 1 µg/ml pepstatin A
  - 5 µg/ml aprotinin
  - 20 µg/ml leupeptin
  - 5 mM iodoacetamide (IAA)
  - 25 mM N-ethylmaleimide (NEM).

3. Preparation of Guanidinium Lysis Buffer Samples

- Centrifuge the remaining 4 ml of the cell suspension at 2000 rpm for 3 minutes.
- Discard the supernatant and resuspend the cell pellet in 5 ml of guanidinium-containing lysis buffer (B1), supplemented with:
  - β-mercaptoethanol
  - Protease inhibitors.
- Store samples at -80°C or immediately subject them to sonication at 4°C.

4. NiNTA Resin Preparation

- Prepare 25 µl of NiNTA resin per sample in a fresh reaction tube.
- Wash resin twice with B1 buffer.

5. Protein Binding

- Add pre-washed NiNTA resin beads to the samples.

- Incubate overnight at 4°C on a spinning shaker.

6. Washing and Elution

- Centrifuge the samples at 4500 rpm and 4°C for 10 minutes.
- Wash the NiNTA resin with 1 ml of B1 buffer and transfer it to a fresh reaction tube.
- Wash the NiNTA resin as follows:
  - Once with B2 buffer.
  - Twice with B3 buffer.
  - Ensure all washing buffers are freshly supplemented with β-mercaptoethanol and protease inhibitors.
- Elute proteins from the NiNTA resin using 20 µl of elution buffer.
- Boil samples at 95°C for 3 minutes.

7. Storage and Analysis

- Store samples at -20°C until they are subjected to SDS-PAGE and immunoblot analysis

B1		
	6 M	Guanidinium/HCl
	0.1 M	Na2HPO4
	0.1 M	NaH2PO4
	10 mM	Tris/HCl pH 8.0
	20 mM	Imidazole
	5 mM	β-mercaptoethanol

B2		
	8 M	Urea
	0.1 M	Na2HPO4
	0.1 M	NaH2PO4
	10 mM	Tris/HCl pH 8.0
	20 mM	Imidazole
	5 mM	β-mercaptoethanol

B3		
	8 M	Urea
	0.1 M	Na2HPO4
	0.1 M	NaH2PO4
	10 mM	Tris/HCl pH 6.3
	20 mM	Imidazole
	5 mM	β-mercaptoethanol

Elution buffer		
	200 mM	Imidazole
	0.1% (v/v)	SDS
	150 mM	Tris/HCl pH 6.3
	30% (v/v)	Glycerol
	720 mM	β-mercaptoethanol
	0.01% (w/v)	Bromphenol blue