**Ubiquitin immunoprecipitation using an anti-ubiquitin nanobody**

**Authors:** Cole S Sitron1, Victoria A Trinkaus1, F Ulrich Hartl1

1Department of Cellular Biochemistry, Max Planck Institute of Biochemistry

**Abstract**

This protocol describes a method to detect ubiquitination on a protein of interest. This technique relies on immunoprecipitation (IP) of ubiquitinated proteins from a cell lysate through the use of an anti-ubiquitin nanobody coupled to agarose beads. The eluate can be run on SDS-PAGE to determine whether the protein of interest was recovered in the IP and, therefore, ubiquitinated.

Keywords: immunoprecipitation, IP, ubiquitin, nanobody

**Materials**

Cell culture

2-4 50-80% confluent wells of cells growing in a 6-well plate.

Equipment

* BioRuptor Plus sonicator (Diagenode cat. no. B01020001) (or equivalent)
* CLARIOstar Plus Plate Reader (BMG Labtech) (or equivalent)
* Microcentrifuge

Specialized Reagents

* Ubiquitin pan-selector resin (NanoTag Biotechnologies cat. no. N2510)
* MiniSpin Columns (NanoTag Biotechnologies cat. no. A1001-L)
* TrypLE Express (Gibco cat. no. 12605036)
* Pierce Rapid Gold BCA Protein Assay Kit (Thermo Fisher Scientific A53225)

Buffers

* Triton Lysis Buffer: 20 mM Tris pH 8, 150 mM NaCl, 1% Triton X-100, 1X cOmplete, Mini EDTA-free Protease Inhibitor Cocktail (Roche cat. no. 11873580001), freshly-added 20 mM N-ethylmaleimide (2.5 mg/ml; Sigma Aldrich E3876-25G), 50 uM PR-619 (from 100 mM DMSO stock; Sigma-Aldrich 662141-25MG), 7.5 U/ml benzonase (Max Planck Institute of Biochemistry Core Facility)
* Triton Wash Buffer: 20 mM Tris pH 8, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 50 uM PR-619
* 4X NuPAGE LDS Sample Buffer (Invitrogen cat. no. NP0007) + 5% beta-mercaptoethanol (Sigma Aldrich cat. no. M6250-100ml)
* 10% FBS (Thermo Fisher Scientific cat. no. 10270106) in 1X PBS (diluted from 10X PBS pH 7.4; Thermo Fisher Scientific cat. no. 70011051)
* 1X PBS

**Cell collection and lysis**

1. Remove the medium from the wells and trypsinize with 500 ul TrypLE Express.
2. Quench the TrypLE Express with 500 ul 10% FBS and move the cells into a centrifuge tube
3. Pellet cells at 1500 x g for 3 min at 4C.
4. Wash cells with 1 ml PBS and pellet again.
5. Resuspend cells in 150 ul Triton Lysis Buffer and incubate on ice for 5 min.
6. Sonicate in the BioRuptor for 5 cycles of 30 seconds on and 30 seconds off at 4C.
7. Clarify the lysate by centrifugation 18000 x g for 10 min 4C.
8. During the centrifugation, prepare BSA standards according to the Rapid Gold BCA Protein Assay Kit manual and begin equilibrating 50 ul of Ubiquitin pan-selector resin (pipette with a cut p200 tip) in 500 ul of lysis buffer.
9. Collect supernatant and place into a new tube on ice.
10. Prepare a small 1:10 dilution of samples and perform Rapid Gold BCA Protein Assay according to manufacturer’s instructions.

**Immunoprecipitation (IP)**

1. Dilute samples into Triton Lysis Buffer in two tubes: 1 mg in 500 ul (for IP) and 100 ug in 25 ul (for input).
2. Pellet the beads at 1000 x g for 2 min at 4C.
3. Pull of the supernatant, add the 1 mg of lysate to the beads, and incubate with rotation at 4C for 1 hr.
4. During the incubation denature the input sample with 25 ul 4X NuPAGE LDS Sample Buffer at 95C 5 min.
5. Pellet the beads at 1000 x g for 2 min at 4C.
6. Wash the beads with either 1 ml Triton Wash Buffer.
7. Pellet the beads and repeat for a total of 3 washes.
8. Remove the supernatant and add 100 ul of 2X NuPAGE LDS Sample Buffer.
9. Elute by boiling at 95C for 5 min.
10. Transfer the beads to a MiniSpin column placed in a tube.
11. Centrifuge 3000 x g for 3 min.
12. The eluate has now been collected in the tube and is ready for SDS-PAGE analysis.