Jun 30, 2025

Ubiquitin enrichment assay

Nature Communications

DOI
https://dx.doi.org/10.17504/protocols.io.eq2ly4z7rlx9/v1

Bishal Basak[1,2], Erika Holzbaur[1,2]

[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104; [2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

University of Pennsylva...

Bishal Basak
University of Pennsylvania

DOI: https://dx.doi.org/10.17504/protocols.io.eq2ly4z7rlx9/v1

Protocol Citation: Bishal Basak, Erika Holzbaur 2025. Ubiquitin enrichment assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2ly4z7rlx9/v1

Manuscript citation:
https://www.biorxiv.org/content/10.1101/2024.06.12.598718v1.full

Protocol status: Working
We use this protocol and it's working

Created: June 30, 2025

Last Modified: June 30, 2025

Protocol Integer ID: 221323

Keywords: ubiquitin enrichment, neuronal lysate, assay

Funders Acknowledgements:
ASAP
Grant ID: ASAP-000350

Abstract

Ubiquitin enrichment assay from neuronal lysates

Protocol materials

Signal-Seeker Ubiquitination Detection Kit **Cytoskeleton Inc.** **Catalog #**BK161

Plate neurons

1 Plate 3- 5 million neurons in a 10 cm dish at days in vitro (DIV) 0

Assay steps

2 When neurons are mature (DIV 6 or more), treat neurons depending on experimental needs

3 Post treatment, aspirate out media, wash neurons once with 1X fresh PBS

4 Lyse neurons in lysis buffer containing 50 mM HEPES (pH=7.4), 1 mM EDTA, 1 mM $MgCl_2$, 25 mM NaCl, 0.5% Triton X-100, 20µg/mL Leupeptin, 2 mM DTT, 20 µg/mL TAME, 2 µg/mL Pepstatin A, 1 mM PMSF. Add Deubiquitination inhibitors provided with Ubiquitin Detection Kit (Cytoskeleton, BK161) to the lysis buffer at 1X concentration.

Signal-Seeker Ubiquitination Detection Kit **Cytoskeleton Inc. Catalog #**BK161

5 Post lysis of the neurons, spin lysates at 17000x g for 10 mins, and discard the pellet.

6 Store 5% of the supernatant as input to be used for western blotting later.

7 Wash Ubiquitination affinity beads and Ubiquitination IP control beads (provided in the kit) with 1XPBST twice and 1XPBS once, and then incubate with equal volumes of the lysate for 4 hrs at 4^0C.

Note

The assay was performed using Signal Seeker Ubiquitination Detection Kit, as mentioned in the manufacturer's protocol with some modifications.

Signal-Seeker Ubiquitination Detection Kit **Cytoskeleton Inc. Catalog #**BK161

8 Post incubation, centrifuge beads at 5000x g at 4^0C for 1 min.

9 Wash beads thrice with BlastR-2^{TM} wash buffer for 5 minutes each in a rotating platform at 4^{0}C and centrifuge at 5000Xg.

10 After final wash and centrifugation, remove buffer supernatant completely.

11 Incubate beads with Bead Elution Buffer for 5 mins at RT and then centrifuge in a spin column at 10000x g for 1 min to collect the immunoprecipitates.

12 Add 2-mercaptoethanol to each sample and boil samples at 95^{0}C for 10 minutes to be used for western blotting.