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# OPEN ACCESS



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

# (3) Ubiquitin immunoprecipitation using an anti-ubiquitin nanobody

Cole S Sitron<sup>1</sup>, Victoria A Trinkaus<sup>1</sup>, F Ulrich Hartl<sup>1</sup>

<sup>1</sup>Department of Cellular Biochemistry, Max Planck Institute of Biochemistry

ASAP Collaborative Research Network



Cole Sitron

#### **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.

#### **ATTACHMENTS**

io\_Ubiquitin immunoprecipitation using an anti.docx

**Created:** Jan 16, 2024

**MATERIALS** 

Last Modified: Jan 22, 2024

**Materials** 

PROTOCOL integer ID: 93785

**Cell culture** 

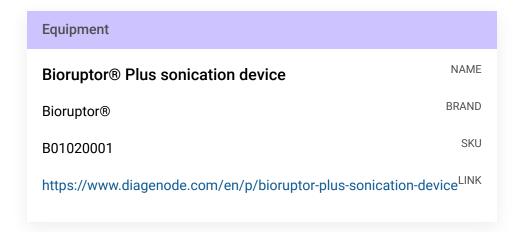
#### **Funders Acknowledgement:**

Aligning Science Across Parkinson's

Grant ID: ASAP-000282

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

### **Equipment**



- CLARIOstar Plus Plate Reader (BMG Labtech) (or equivalent)
- Microcentrifuge

#### **Specialized Reagents**

- W Ubiquitin Selector NanoTag Biotechnologies Catalog #N2510
- MiniSpin Columns (50 units) NanoTag Biotechnologies Catalog #A1001-L
- TrypLE™ Express Enzyme (1X), phenol red **Thermo**Fisher Catalog #12605036
- Pierce™ Rapid Gold BCA Protein Assay Kit **Thermo**Fisher Catalog #A53225

### **Buffers**

Triton Lysis Buffer:

А	В
Tris pH 8	20 mM
NaCl	150 mM
Triton X-100	1%
cOmplete, Mini EDTA-free Protease Inhibitor Cocktail (Roche cat. no. 11873580001)	1X
Freshly-added 20 mM N-ethylmaleimide (2.5 mg/ml; Sigma Aldrich E3876-25G)	2.5 mg/ml
50 uM PR-619 (from 100 mM DMSO stock; Sigma- Aldrich 662141-25MG)	50 uM
Benzonase (Max Planck Institute of Biochemistry Core Facility)	7.5 U/ml

- 🔀 cOmplete™ EDTA-free Protease Inhibitor Cocktail Roche Catalog #11873580001
- N-Ethylmaleimide Merck MilliporeSigma (Sigma-Aldrich) Catalog #E3876
- DUB Inhibitor V, PR-619 Merck MilliporeSigma (Sigma-Aldrich) Catalog #662141

#### Triton Wash Buffer:

	A	В
	Tris pH 8	20 mM
	NaCl	150 mM
Г	EDTA	1 mM
Г	Triton X-100	1%
Г	SDS	0.1%
	PR-619	50 uM

4X

NuPAGE™ LDS Sample Buffer (4X) **Thermo Fisher Scientific Catalog #NP0007** 

5% beta-mercaptoethanol

2-Mercaptoethanol Merck MilliporeSigma (Sigma-Aldrich) Catalog #M6250

-100ml)

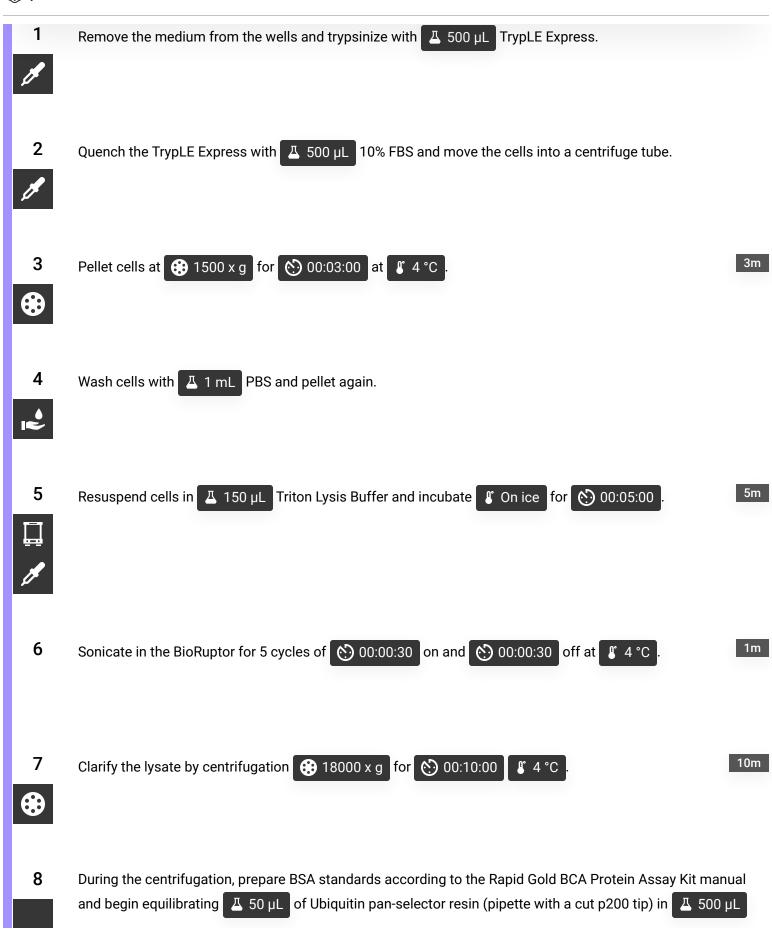
■ 10% FBS ( Fetal Bovine Serum Gibco - Thermo Fischer Catalog #10270106 
1X PBS (diluted from

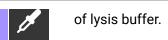
PBS (10X), pH 7.4 Thermo Fisher Scientific Catalog #70011051

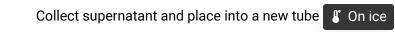
Oct 22 2024

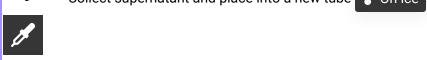
1X PBS

# **Cell collection and lysis**

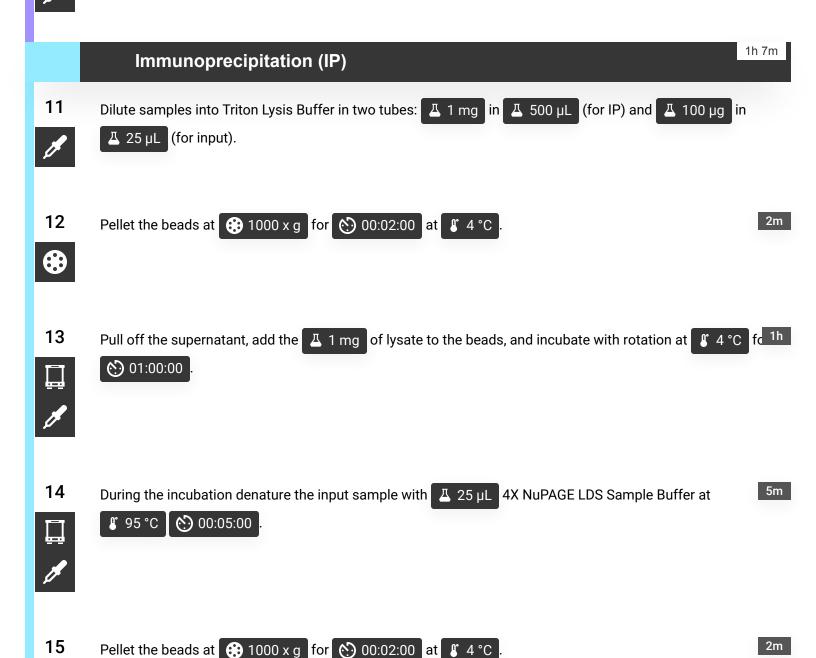








Prepare a small 1:10 dilution of samples and perform Rapid Gold BCA Protein Assay according to manufacturer's instructions.





16 Wash the beads with either 4 1 mL Triton Wash Buffer.



17 Pellet the beads and repeat for a total of 3 washes.



18 Remove the supernatant and add Δ 100 μL of 2X NuPAGE LDS Sample Buffer.



Elute by boiling at \$\mathbb{8} 95 \cdot \text{for } \cdot 00:05:00

5m

Transfer the beads to a MiniSpin column placed in a tube.



**(B)** 

21 Centrifuge 3000 x g for 00:03:00

3m

The eluate has now been collected in the tube and is ready for SDS-PAGE analysis.

