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Immunoprecipitation of NAP1-GFP

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

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Abstract

This protocol describes immunoprecipitation of NAP1-GFP from HAP1 cells.

Attachments

PDF

757-1925.pdf

43KB

Materials

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- NAP1-EGFP (Addgene xxx)
- Lipofectamine 2000 (Thermo Fisher)
- SDS-PAGE gels (NP0322BOX, Thermo Fisher)
- PageRuler Prestained protein marker (Thermo Fisher)

Lysis buffer

	A	B
	KCl	100 mM
	$MgCl_2$	2.5 mM
	Tris-HCl pH 7.4	20 mM
	NP-40	0.50%

Fixation solution

	A
	40% ethanol
	10% acetic acid
	50% dH2O

Pierce™ Detergent Compatible Bradford Assay Kit **Thermo Fisher Catalog #**23246

ChromoTek GFP-Trap® Agarose **Proteintech Catalog #** gta-20

Immunoprecipitation

1h

1 Transiently transfect FIP200 knockout HAP1 cells with pcDNA3.1 NAP1-EGFP (Addgene), or empty pcDNA3.1 vector as a negative control, using Lipofectamine 2000 (Thermo Fisher).

> Note
>
> This cell line was selected as FIP200 deletion results in TBK1 hyperactivation and thus increased NAP1 phosphorylation.

2 After 48 h, collect the cells by trypsinization and wash the cell pellet with PBS once. Then, lyse the cells in lysis buffer.

3 Lyse the samples for 00:20:00 On ice . Then, clear the cell lysates by centrifugation at 20000 x g, 4°C, 00:10:00 . 30m

4 Determine the protein concentrations of the cleared protein lysates with the Pierce Detergent Compatible Bradford Assay Kit (23246, Thermo Fisher).

5 For both samples, negative control and NAP1-EGFP lysates, incubate 12 mg of cell lysate Overnight with 20 µL or GFP-Trap agarose beads (GTA-20, Chromotek). 20m

6 In the morning, wash the samples three times in lysis buffer. Then, resuspend the beads in protein loading dye, supplemented with 100 mM DTT. Finally, boil the samples for 00:05:00 at 95 °C . 5m

7 Load the samples on 4-12% SDS-PAGE gels (NP0322BOX, Thermo Fisher) with PageRuler Prestained protein marker (Thermo Fisher).

8 After the run, stain the SDS-PAGE gel with Coomassie, fix for 00:10:00 in fixation solution, and destain it Overnight in dH2O. 15m

9 Cut the band corresponding to NAP1-EGFP from the gel with a fresh scalpel and submit for mass spectrometry analysis.