Co-immunoprecipitation using GFP-trap

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JUL 25, 2023

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.6qpvr36o2vmk/v1

Protocol Citation: Dan Dou, Erika Holzbaur 2023. Co-immunoprecipitation using GFP-trap. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvr36o2vmk/v1

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

Created: Jul 20, 2023

Last Modified: Jul 25, 2023

PROTOCOL integer ID: 85310

Keywords: ASAPCRN

ABSTRACT

Here, we describe performing co-immunoprecipitation experiments in HEK293 cells using GFP-trap beads

1 See "Protocol: HEK293 cell culture for co-immunoprecipitation experiments" for preceding culture and transfection. For the present study, HEK cells were transfected with EGFP-tagged

bait proteins. Prey proteins were either endogenous or transfected with HA- or SNAP-tags.

2 Prepare wash buffer:

	A	B
	Tris-HCl pH 7.5	10 mM
	NaCl	150 mM
	EDTA	0.5 mM
	Triton-X (optional)	0.4%

Note

Triton-X should be included for prey proteins where appreciable non-specific binding is observed in the EGFP vector condition.

3 Prepare lysis buffer. Lysis buffer composition differed for experiments needing lambda phosphatase treatment

3.1 Lysis buffer for non-lambda phosphatase experiment:

	A	B
	Tris-HCl pH 7.5	10 mM
	NaCl	150 mM
	EDTA	0.5 mM
	NP-40	0.5%
	PMSF	1 mM
	TAME	0.01 mg/mL
	Leupeptin	0.01 mg/mL
	Pepstatin A	0.001 mg/mL

3.2 Lysis buffer compatible with lambda phosphatase experiment:

	A	B
	1x NEBuffer for Protein MettaloPhosphatases (New England BioLabs)	1x
	NP-40	0.5%
	Leupeptin	0.01 mg/mL
	ddH2O	To desired volume

4 24 hours after transfection, wash HEK cells twice in ice-cold PBS and lyse in appropriate lysis buffer. Use 600 µL lysis buffer per experimental condition (pooled across the three 10cm dishes).

5 Clarify lysates at 10 x g at 4 degrees C for 10 minutes

6 Wash 25 µL GFP-trap beads per experimental condition in 500 µL wash buffer, in low protein binding Eppendorf tubes under rotating agitation.

> Note
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> For large protein complexes, GFP-Trap Magnetic Particles M-270 should be used instead of GFP-Trap Magnetic Agarose beads

7 Equilbrate beads in lysis buffer for 5 min at 4 degrees C under rotating agitation

8 For lambda phosphatase experiments: add 60 µL of 10 mM $MnCl_2$ and 24 µL lambda phosphatase (2,400 units; New England BioLabs) for a final reaction volume of 600 µL per experimental condition.

Note

For conditions that are lambda phosphatase-negative, add 24 µL ddH_2O instead

8.1 Incubate at 30 degrees C for 30 minutes

9 Incubate beads with clarified lysate (or lambda phosphatase-treated lysate) for 1 hour at 4 degrees C under rotating agitation

10 Wash beads three times for 5 min in wash buffer at 4 degrees C under rotating agitation

11 Resuspend beads in 60 µL denaturing buffer, and boil for 10 minutes to release bound protein