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Immunoprecipitation

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We use this protocol and it's working

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Abstract

This protocol details the immunoprecipitation of Hela cells.

Materials

1% Triton X100 lysis buffer

	A	B
	1% Triton X-100 Buffer:	Amount/Liter
	Tris, 50 mM	6.057 g
	NaCl, 150 mM	8.766 g
	Triton X-100, 1%	10.0 ml

Pierce Bradford Assay reagent

	A	B	C	D
	Coomassie Reagent (ml)	BSA (2 µg/µl) volume (µl)	BSA concentration (µg/µl)	Sample (µl)
	1	0	0	0
	1	1	2	0
	1	2	4	0
	1	4	8	0
	1	8	16	0
	1			2

Pierce™ Coomassie (Bradford) Protein Assay Kit **Thermo Fisher Catalog #**23200

10 x Sample buffer

	A	B
	Chemical	Amount/20 ml
	0.5 M Tris pH 6.8	10 ml
	10% SDS	2 g
	50% Sucrose	10 g
	0.04% Bromophenol Blue	8 mg
	1.2 M Mercaptoethanol	2 ml

1 Seed Hela cells into 150 mm dishes at a density of 1.9 million cells. The cells will be near confluent in 2 days. Otherwise seeding 4x10^6 of cells/150 mm dish will be near confluent the next day. Expect to get 3- 5 mg of protein per dish. These numbers will be different for other cell lines and will have to be determined prior to the experiment.

2 Turn on the centrifuge, install rotor for 1.5 ml tubes and chill to 4 °C .

3 To 1% Triton X100 lysis buffer add Roche Complete Mini, EDTA-free protease inhibitor (1 tablet/10 ml) and Roche PhosSTOP phosphatase inhibitor (1 tablet/10 ml). Allow to dissolve, vortexing occasionally.

	A	B
	1% Triton X-100 Buffer:	Amount/Liter
	Tris, 50 mM	6.057 g
	NaCl, 150 mM	8.766 g
	Triton X-100, 1%	10.0 ml

Adjust pH to pH 7.4

4 Prepare ice, sample tubes, PBS for washing cells, cell scrapers and a beaker for decanting media.

5 Decant media into a beaker and wash cells 3x with 20 mL of ice-cold PBS/150 mm dish, each time decanting into the beaker. After the last wash aspirate off as much media as possible.

6 Place cell culture dish On ice , add 1 mL /150 mm dish of 1% Triton X100 + dissolved protease inhibitors, scrape the cells off using a cell scraper and transfer the cell mixture into a chilled microcentrifuge tube. If using several dishes, harvest one dish and then transfer the lysate to the next dishes to minimize the volume.

7 Spin lysates for 20000 x g, 4°C, 00:05:00 . Collect the supernatant and place in new chilled microcentrifuge tubes.

5m

8 Prepare standards and samples as indicated in the table for analysis of protein concentrations using the Pierce Bradford Assay reagent (ThermoFisher Scientific catalog # 23200).

	A	B	C	D
	Coomassie Reagent (ml)	BSA (2 µg/µl) volume (µl)	BSA concentration (µg/µl)	Sample (µl)
	1	0	0	0
	1	1	2	0
	1	2	4	0
	1	4	8	0
	1	8	16	0
	1			2

9 Measure protein concentration using the NanoDrop or other spectrophotometer.

10 For immunoprecipitation, prepare the Chromotek GFP-TRAP agarose resin by aliquoting 10 µL of bead suspension per sample. Make sure that the beads are well resuspended before pipetting. To facilitate pipetting cut off the ends of the micropipette tips. 20 µL of unconjugated agarose beads can be added per sample to help with visualizing the bead pellet.

11 Wash beads 3x with 1 mL lysis buffer. For each wash invert tube up and down a few times, pellet the resin by centrifugation for 10.000 x g, 00:00:30 and carefully aspirate the supernatant. 30s

12 After the last wash resuspend the resin in a small volume of lysis buffer and divide it equally between sample tubes. Spin again and aspirate off excess lysis buffer.

13 Transfer cell lysates into the tubes containing resin (use equal protein amounts for each sample typically ~ 1 mL of 3- 5 undetermined lysate). Save 10% of total cell lysate to use as a control during immunoblotting.

14 Rotate immunoprecipitation samples end-over-end at 4 °C for 01:00:00 . 1h

15 At the end of the incubation centrifuge 10000 x g, 00:00:30 to collect beads; aspirate most of supernatant using a vacuum and super fine tip, being careful not to disrupt the pellet. 30s

16 Wash pelleted beads 4-6x with lysis buffer (1 mL for each wash). For each wash, mix the suspension for ~ 00:00:03 , spin down (~ 00:00:10 pulse), and aspirate supernatant. 13s

To be safe, do not try to get all of it -- leave some (5%) buffer over the beads. Work quickly and keep samples cold.

17 Using a fine pipette tip, remove remaining supernatant from the beads after last wash. Place tubes back On ice .

18 Add 50 µL of 2X sample buffer to each tube of beads; vortex at ~1/2 speed, keeping beads in bottom of tube.

10 x Sample buffer

	A	B
	Chemical	Amount/20 ml
	0.5 M Tris pH 6.8	10 ml
	10% SDS	2 g
	50% Sucrose	10 g
	0.04% Bromophenol Blue	8 mg
	1.2 M Mercaptoethanol	2 ml

19 Denature samples at 95 °C for 00:03:00 .

3m

20 Centrifuge at 10000 x g, 00:00:30 .

30s

21 Transfer supernatant to new tube using a fine pipette tip. (You can pick up residual sample from the beads by pressing the fine pipette tip against bottom of tube and rocking it back and forth, excluding beads).

22 Samples may be frozen at this point, or you can proceed to immunoblotting.