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Magnet-assisted immunoprecipitation (MAIP) of recombinant protein

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We use this protocol and it's working

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Abstract

This protocol describes a magnet-assisted immunoprecipitation (MAIP) workflow for the isolation of a TGF-β1/PD-L1 fusion protein from mammalian cell lysates under native conditions. Dynabeads M-280 Tosylactivated are covalently coupled with either anti-TGF-β1 or anti-PD-L1 antibodies to enable selective capture of the fusion protein following transient expression in HEK 293 cells. The method includes detailed steps for antibody coupling, protein extraction, immune-magnetic isolation, acidic elution, and neutralization, allowing recovery of intact protein suitable for downstream biochemical analyses such as SDS-PAGE and immunoblotting.

Magnetic beads preparation

2d 17h 9m 40s

1 Prepare the magnet-coated beads. For this protocol, the Dynabeads M-280 Tosylactivated beads are used 2d

1.1 Resuspend the Dynabeads in the vial by vortexing for 00:00:30 30s

1.2 Transfer 300 µL of the beads to a new, sterile 1.5 mL tube

1.3 Add 1 mL of Buffer B to the tube and vortex for 00:00:30 30s

	A	B	C
	Reagent	**Molecular weight (g/mol)**	**Amount needed**
	NaH2PO4 × H2O	137.99	2.62 g
	Na2 HPO4 × 2 H2O	177.99	14.42 g
	NaOH or HCl	–	pH 7.4
	Distilled water (H2O)	18.015	Fill to 1 L

Buffer B composition

1.4 Place the tube in a magnet for 00:01:00 and discard the supernatant. Repeat go to step #1.3 once 1m

1.5 Remove the tube from the magnet and resuspend the washed beads in 300 µL of Buffer B. Divide the washed beads into two tubes, containing 150 µL each

2 Couple the antibodies covalently with Dynabeads M-280 Tosylactivated using the recommended protocol from the manufacturer. For this protocol, the anti-TGF-β1 and

anti-PD-L1 antibodies will be used. However, different antibody reagents can be subjected for coupling

2.1 Place the tubes in a magnet for 00:01:00 and discard the supernatant 1m

2.2 For every 150 µL of Dynabeads as the initial volume, resuspend in 100 µg/150 µL of antibodies (anti-TGF-β1 or anti-PD-L1) diluted using Buffer B. Mix by vortexing for 00:00:30 30s

2.3 Add 100 µL Buffer C and mix by vortexing for 00:00:30

	A	B
	Reagent	**Amount needed**
	[(NH4)2]SO 4	39.64 g
	Buffer B	100 mL
	NaOH or HCl	pH 7.4

Buffer C composition

2.4 Incubate on a shaker at 37 °C for 16:00:00 16h

2.5 Place the tube on a magnet for 00:02:00 and remove the supernatant 2m

2.6 Remove the tube from the magnet and add 1 mL Buffer D; incubate at 37 °C for 01:00:00 on a shaker. 1h

	A	B
	Reagent	**Amount needed**
	NaCl	0.88 g

	A	B
	BSA (Bovine Serum Albumin)	0.5% (w/v)
	Buffer B	80 mL
	NaOH or HCl	pH 7.4

Buffer D composition

2.7 Place the tube on a magnet for 00:02:00 and remove the supernatant. 2m

2.8 Remove the tube from the magnet and add 1 mL Buffer E; vortex for 00:00:10 10s

2.9 Place the tube on a magnet for 00:02:00 and remove the supernatant. 2m

2.10 Repeat steps go to step #2.7 and go to step #2.8 once.

2.11 For every 150 µL of Dynabeads, resuspend and dilute the beads in 200 µL of Buffer E.

	A	B
	Reagent	**Amount needed**
	NaCl	0.88 g
	BSA (Bovine Serum Albumin)	0.1% (w/v)
	Buffer B	80 mL
	NaOH or HCl	pH 7.4

Buffer E composition

Protein extraction

2d 0h 11m

3 Following transfection, harvest HEK 293 cells after 48:00:00 of incubation. 2d

3.1 Decant the culture medium and wash the cells twice with sterile PBS.

3.2 Submerge the cells in PBS and incubate at 25 °C for 00:01:00 1m

3.3 Tap the sides of the plate gently to detach the cells and transfer the supernatant.

3.4 Collect the cells by centrifugation at 500 x g, 25°C, 00:05:00 5m

4 For every 1 × 10^6 cells, resuspend using 500 µL of native protein extraction buffer.

	A	B
	Reagent	**Amount needed**
	Tris	50 mM
	EDTA	1 mM
	SDS	0.001% (w/v)
	Tween-20	0.01% (w/v)
	NaCl	50 mM

Composition of the native protein extraction buffer, pH 7.4

5 Lyse the cells by passing the solution through a 23-G needle syringe 15 times. This should be done gently and on ice.

6 Clarify the lysate by centrifugation at 500 x g, 25°C, 00:05:00. Transfer the supernatant to a new sterile tube, and store the pellet at -80 °C for future protein recovery. 5m

7 Quantify the protein content of the extract. Any protein quantification method available in the laboratory may be used.

Immuno-magnetic isolation

37m 10s

8 Add 10 µg of sample containing the recombinant protein to 50 µL of the antibody-coupled beads. For this protocol, a bifunctional fusion protein that contains the TGF-B1 and PD-L1 domains are used. Prepare two separate reactions for each antibody-coupled Dynabeads (anti-TGF-β1 or anti-PD-L1)

9 Incubate with tilting and rotation for 00:30:00 30m

10 Place the tube on a magnet for 00:02:00 and pipette off the supernatant 2m

11 Remove the tube from the magnet and add 1 mL PBS; vortex for 00:00:10 10s

12 Place the tube on the magnet for 00:02:00 and remove the supernatant 2m

13 Repeat steps go to step #11 and go to step #12 twice

14 Add 50 µL of 100 mM glycine-HCl (pH 3.0) and incubate for 00:01:00 1m

15 Place the tube on the magnet for 00:02:00 and collect the supernatant 2m

16 Neutralize the collected supernatant by adding 5 µL of 1 M Tris-HCl (pH 8.8). At this point, the beads may also be washed with 50 µL of 1 M Tris-HCl for reuse in future isolations.

17 Quantify the concentration of the isolated protein and analyze the purity using SDS-PAGE or other methods