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Coupling of TMEM192 antibody to MyOne™ Epoxy Dynabeads™

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We use this protocol and it's working

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Abstract

This protocol describes coupling of 600 µg of rabbit monoclonal TMEM192 antibody (Abcam recombinant Anti-TMEM192 antibody [EPR14330-67], BSA and Azide free, ab232600) to 20 mg of MyOne™ Epoxy Dynabeads™ (Invitrogen™, 34001D) to obtain 2 mL of final suspension. The coupled beads generated using this protocol can be used for the isolation of untagged lysosomes from cells and tissues.

Attachments

PDF

230607-Dynabeads_cou...

130KB

Guidelines

™

Note

This protocol can be used for coupling of any monoclonal antibody to MyOne™ Epoxy Dynabeads. Antibody to be coupled should be provided in PBS at a concentration of at least 1.2 mg/ml, and must be free of sodium azide, amine-based buffers, glycerol and protein stabilisers (BSA). Maximum binding capacity of MyOne™ Epoxy Dynabeads™ is estimated to be ~ 20 µg - 30 µg of antibody per mg of beads. In this protocol 30 µg per mg of beads is used, to ensure complete saturation of beads.

Materials

Buffers:

	A	B
	C1	0.1 M Sodium Phosphate (Na2HPO4:NaH2PO4) buffer pH 7.4
	C2	3 M Ammonium Sulphate ((NH4)2SO4) in 0.1 M Sodium Phosphate buffer pH 7.4
	HB	100 mM Glycine pH 11.3, 0.01% Tween-20
	LB	200 mM Glycine pH 2.8, 0.01% Tween-20
	SB	50 mM Tris-HCl (NH2C(CH2OH)3·HCl) pH 7.4 with 140 mM NaCl and 0.1% Tween-20
	SBS	50 mM Tris-HCl (NH2C(CH2OH)3·HCl) pH 7.4 with 140 mM NaCl, 0.1% Tween-20 and 0.2% NaN3

Note

All buffers should be stored at 4 °C (up to 1 week) or at -20 °C (long-term storage) and must be brought to Room temperature before being used for coupling.

Troubleshooting

Protocol

1d 0h 11m 15s

1 Before opening the vial containing dried magnetic beads, equilibrate to Room temperature.

2 Thaw the antibody On ice and keep On ice until it is needed in step 8.

3 Calculate the volume of antibody needed, so that 600 µg is used – this volume should be ≤ 500 µL.

4 Weigh 20 mg beads directly into a fresh low-binding 1.5 ml Eppendorf tube.

5 Resuspend beads in 1000 µL of sterile Milli-Q water, vortex for 00:00:15, sonicate in a water bath sonicator for 00:05:00. 5m 15s

6 Place vial on a magnetic rack for 00:01:00, remove water using a pipette. 1m

7 Repeat steps 5 and 6. After sonication there should be no bead aggregates visible.

8 Add the required volume of antibody to the vial containing washed beads.

9 Add buffer C1 up to total volume of 500 µL (C1= 500 - antibody volume). Vortex to resuspend the beads.

10 Add 500 µL of buffer C2 and vortex.

11 Incubate in a Thermomixer at 37 °C for 16–24 hours (typically 20:00:00) at 1500 rpm (make sure the beads do not settle). 20h

12 Place on a magnetic rack for 00:01:00, remove liquid using a pipette. 1m

13 Resuspend beads in 1000 µL of buffer HB, vortex.

14 Place on a magnetic rack for 00:01:00, remove liquid using a pipette. 1m

15 Resuspend beads in 1000 µL of buffer LB, vortex.

16 Place on a magnetic rack for 00:01:00, remove liquid using a pipette. 1m

17 Resuspend beads in 1000 µL of buffer SB, vortex.

18 Repeat steps 16 and 17:
- Place on a magnetic rack for 00:01:00, remove liquid using a pipette.
- Resuspend beads in 1000 µL of buffer SB, vortex.

1m

19 Place on a magnetic rack for 00:01:00, remove liquid using a pipette. 1m

20 Resuspend beads in 1000 µL of buffer SB, vortex.

21 Incubate in a shaker at Room temperature for 1500 rpm, 00:15:00

22 Place on a magnetic rack for 00:01:00, remove liquid using a pipette. 1m

23 Resuspend beads in 1000 µL of buffer SBS. At this stage beads are at 20 mg/ml and should be stored in the fridge. Beads can be further diluted with buffer SBS to 10 mg/ml, which is the usual working concentration.