Coupling of TMEM192 antibody to MyOne™ Epoxy Dynabeads™

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.

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 ~20-30 µg of antibody per mg of beads. In this protocol 30 µg per mg of beads is used, to ensure complete saturation of beads.

Buffers:

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% NaN_3

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 RT before being used for coupling.

Protocol:

1. Before opening the vial containing dried magnetic beads, equilibrate to RT.
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 15 s, sonicate in a water bath sonicator for 5 minutes.
6. Place vial on a magnetic rack for 1 min, remove water using a pipette.
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 hours) at 1500 rpm (make sure the beads do not settle).
12. Place on a magnetic rack for 1 min, remove liquid using a pipette.
13. Resuspend beads in 1000 µl of buffer HB, vortex.
14. Place on a magnetic rack for 1 min, remove liquid using a pipette.
15. Resuspend beads in 1000 µl of buffer LB, vortex.
16. Place on a magnetic rack for 1 min, remove liquid using a pipette.
17. Resuspend beads in 1000 µl of buffer SB, vortex.
18. Repeat steps 16 and 17.
19. Place on a magnetic rack for 1 min, remove liquid using a pipette.
20. Resuspend beads in 1000 µl of buffer SB, vortex.
21. Incubate in a shaker at RT for 15 minutes at 1500 rpm.
22. Place on a magnetic rack for 1 min, remove liquid using a pipette.
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.