

Protocol for Antibody BMB Coupling

- 1- Vortex 1.0 ml BMB. (approx. 5,000 beads)
- 2- Wash twice with 1,000 μ l MES 50mM, pH 6.3. (Activation Buffer)
Wash: quick spin and apply to a magnetic stand. Discard the residual buffer.
- 3- Activate BMB by adding 1,000 μ l of activation buffer and add 30 μ l of freshly prepared Sulfo-NHS solution (50mg/ml in Activation Buffer), and 60 μ l of a freshly prepared EDC solution (50mg/ml in Activation Buffer). Incubate on a rotator for 20min.
- 4- Wash three times with 1,500 μ l Coupling Buffer (MES 50mM pH7.5).
- 5- Add 450 μ l of capture Antibody (diluted in coupling Buffer at ~0.1mg/ml, depending on the Ab) 2 hours or preferably one night at Room temperature on a rotator.
- 6- Add 9 μ l of ethanolamine diluted 10X in PBS. Incubate 30min on a rotator
- 7- Wash once with 1,000 μ l wash buffer (PBS 0.5% Tween)
- 8- Resuspend in 1,000 μ l of PBS, 0.5%Tween 20. 10 min at room temperature, with rotation
- 9- Wash once in 1,000 μ l PBS 1%BSA
- 10- Block in 1000 μ l PBS 1%BSA, 30min room temperature, on a rotator.
- 11- Wash twice in PBS 1%BSA 0.1%Azide
- 12- Store in PBS 1%BSA 0.1%Azide (1.0ml)