xMAP® AbC Kit Quick Guide

This Quick Guide is a checklist of all of the steps for performing one coupling reaction using the xMAP Antibody Coupling kit (40-50016) and is not intended to replace the kit user manual (89-00002-00-319). Please refer to the user manual for complete instructions, warnings, and precautions.

Reagent Prep:

1. ☐ Allow kit to equilibrate to room temperature for 20 to 30 minutes

Reagent Calculations:

2. ☐ Calculate the volumes required for each reagent:

a. **Beads** for coupling reaction: Desired number of **Beads** to couple: _____x10^6 Concentration of stock vial: _____x10^6 **Beads**/mL $\text{Volume of stock needed} = \frac{(\#\text{ of beads to couple})}{(\text{conc of stock vial})}$ $\text{Volume of stock needed} = \frac{(\_\_\_\_\_\text{beads})}{(\_\_\_\_\_\ \text{beads}/\text{mL})}$	**Volume of Bead stock needed for the coupling reaction (Step 4):** _____**mL**
b. **Activation Buffer** for Activation step: i. 400 µL for reactions of more than 5x10^6 **Beads** ii. 480 µL for reactions of 5x10^6 **Beads** or less	**Volume of Activation Buffer needed for activation (Step 8):** _____**µL**
c. **Sulfo-NHS** for Activation step: i. 50 µL for reactions of more than 5x10^6 **Beads** ii. 10 µL for reactions of 5x10^6 **Beads** or less	**Volume of Sulfo-NHS needed for activation (Step 11):** _____**µL**
d. **EDC** for Activation step: i. 50 µL for reactions of more than 5x10^6 **Beads** ii. 10 µL for reactions of 5x10^6 **Beads** or less	**Volume of EDC needed for activation (Step 14):** _____**µL**
e. **Antibody** for coupling step: Desired number of **Beads** to couple: _____x10^6 Desired **Antibody** concentration: _____µg/1x10^6 **Beads** Stock **Antibody** concentration: _____µg/mL $\text{Volume of Ab needed} = \frac{(\#\text{ of beads to couple})(\text{Desired Ab conc})}{\text{Stock Ab conc}}$ $\text{Volume of Ab needed} = \frac{(\_\_\_\times 10^6\ \text{beads})\left(\frac{\_\_\_\ \mu\text{g}}{1\times 10^6\ \text{beads}}\right)}{\left(\_\_\_\ \mu\text{g}/\text{mL}\right)\left(\frac{1\ \text{mL}}{1000\ \mu\text{L}}\right)}$	**Volume of Antibody needed for coupling (Step 21):** _____**µL**
f. **Activation Buffer** for coupling step: **Total reaction volume**: _____µL i. 1000 µL for reactions of more than 5x10^6 **Beads** ii. 500 µL for reactions of 5x10^6 **Beads** or less Volume of Activation Buffer needed = **total reaction volume** – volume of **Antibody** Volume of Activation Buffer needed = _____µL - _____µL	**Volume of Activation Buffer needed for coupling (Step 20):** _____**uL**

Microsphere Wash #1:

3. ☐ Resuspend the stock **Beads**
   a. 1 mL – vortex and sonicate for 10 seconds
   b. 4 mL – rotate for 15 minutes at 15-30 rpm
4. ☐ Dispense the desired volume of **Beads** (from **Step 2a**) to the reaction tube
5. WASH STEP – use one disposable pipette per reaction tube
   a. ☐☐ Place reaction tube into magnetic separator (1-2 min)
   b. ☐☐ Remove supernatant with transfer pipette
   c. ☐☐ Add 500 µL of **Activation Buffer** into reaction tube
   d. ☐☐ Vortex and sonicate reaction tube for 10 seconds
6. ☐ Repeat WASH STEP, for a total of two washes
7. ☐ Remove liquid from **Beads** w/ magnetic separator (1-2 min) and disposable pipette

Activation:

8. ☐ Add **Activation Buffer** to reaction tube (from **Step 2b**)
9. ☐ Vortex and sonicate reaction tube for 10 seconds
10. ☐ Vortex **Sulfo-NHS** tube for 10 seconds
11. ☐ Add **Sulfo-NHS** to reaction tube (from **Step 2c**)
12. ☐ Add 250 µL of **Activation Buffer** to 10 mg vial of **EDC**.
13. ☐ Invert **EDC** vial and vortex for 10-12 seconds
14. ☐ Add **EDC** solution to reaction tube (from **Step 2d**)
15. ☐ Vortex and sonicate reaction tube for 10 seconds

Incubation #1:

16. ☐ Shield from light and rotate the reaction tube for 20 ± 2 minutes @ 15-30 rpm
    a. Incubation start time: _____
    b. Incubation end time: _____

Microsphere Wash #2:

17. WASH STEP – use one disposable pipette per reaction tube
    a. ☐☐☐ Place reaction tube into magnetic separator (1-2 min)
    b. ☐☐☐ Remove supernatant with transfer pipette
    c. ☐☐☐ Add 500 µL of **Activation Buffer** into reaction tube
    d. ☐☐☐ Vortex and sonicate reaction tube for 10 seconds
18. ☐ Repeat WASH STEP, for a total of three washes
19. ☐ Remove liquid from **Beads** w/ magnetic separator (1-2 min) and disposable pipette

Coupling:

20. ☐ Add **Activation Buffer** to the reaction tube (from **Step 2f**)
21. ☐ Add **Antibody** to the reaction tube (from **Step 2e**)
22. ☐ Vortex the reaction tube for 10 seconds

Incubation #2:

23. ☐ Shield from light and rotate the reaction tube for 2 hours ± 5 minutes @ 15-30 rpm
    a. Rotation start time: _____
    b. Rotation end time: _____

Microsphere Wash #3:

24. WASH STEP – use one disposable pipette per reaction tube
    a. ☐☐☐ Place reaction tube into magnetic separator (1-2 min)
    b. ☐☐☐ Remove supernatant with transfer pipette
    c. ☐☐☐ Add 500 µL of **Wash Buffer** into reaction tube
    d. ☐☐☐ Vortex and sonicate reaction tube for 10 seconds
25. ☐ Repeat WASH STEP, for a total of three washes
26. ☐ Remove liquid from **Beads** w/ magnetic separator (1-2 min) and disposable pipette
27. ☐ Add 1mL of **Wash Buffer** to the reaction tube
28. ☐ Vortex and sonicate reaction tube for 10 seconds
29. ☐ Protect from light and store at 2-8 ºC until needed