

SAMPLE PROTOCOL FOR HYBRIDIZATION TO FlexMAP UNIVERSAL ARRAY MICROSPHERES – WASHED ASSAY FORMAT

MATERIALS

- Carboxylated fluorescent microspheres with covalently attached anti-TAG sequences
- Biotin-labeled targets with appropriate TAG sequence modification
- 2X T_m Hybridization Buffer – 0.4 M NaCl, 0.2 M Tris, 0.16% Triton X-100, pH 8.0
- 1X T_m Hybridization Buffer – 0.2 M NaCl, 0.1 M Tris, 0.08% Triton X-100, pH 8.0
- Streptavidin-R-phycoerythrin (see REAGENTS list)
- 96 well V-bottom PCR plate and cover (see CONSUMABLES list)
- Pipettors, tips, microfuge tubes, plates, etc. (see CONSUMABLES list)

PROCEDURE

Microspheres should be protected from prolonged exposure to light throughout this procedure.

1. Select the appropriate FlexMAP microsphere sets and resuspend by vortex and sonication for approximately 20 seconds.
2. Combine 2500 microspheres of each set per reaction.
3. Concentrate the FlexMAP microsphere mixture by centrifugation at $\geq 8000 \times g$ for 1-2 minutes.
4. Remove the supernatant and resuspend to 100 of each microsphere set per μL in 2X T_m Hybridization Buffer by vortex and sonication for approximately 20 seconds. (Note: 25 μL are required for each reaction.)
5. Aliquot 25 μL of the FlexMAP microsphere mixture to each well.
6. Add 25 μL of dH₂O to each background well.
7. Add 5 to 25 μL of biotinylated, TAGged targets to the sample wells. (Note: For synthetic biotin-TAG oligonucleotide targets, use 5 to 200 femtomoles per reaction.)
8. Adjust the total volume to 50 μL by adding the appropriate volume of dH₂O to each sample well.
9. Cover the plate to prevent evaporation and denature at 96°C for 90 seconds. *
10. Hybridize at 37°C for 30-60 minutes. *
11. Pellet the FlexMAP microspheres by centrifugation at $\geq 2250 \times g$ for 3 minutes and remove the supernatant. See **Technical Note**.
12. Resuspend the pelleted FlexMAP microspheres in 75 μL of 1X T_m Hybridization Buffer.
13. Pellet the FlexMAP microspheres by centrifugation at $\geq 2250 \times g$ for 3 minutes and remove the supernatant.
14. Repeat steps 12. and 13. for a total of two washes.
15. Resuspend the pelleted FlexMAP microspheres in 75 μL of 1X T_m Hybridization Buffer containing 2 $\mu\text{g}/\text{mL}$ streptavidin-R-phycoerythrin.
16. Incubate at 37°C for 15 minutes.

17. Analyze 50 μ L at 37°C on the Luminex analyzer according to the system manual.

* *These steps can be performed on a thermal cycler programmed as follows –*

Hold at 96°C, 90 seconds

Hold at 37°C, FOREVER

Technical Note: Alternatively, pre-wet a 1.2 μ m Millipore filter plate with 1X Tm Hybridization Buffer and filter by vacuum manifold. Transfer the reactions to the pre-wetted filter plate and remove the supernatant by vacuum filtration. Wash twice with 100 μ L 1X Tm Hybridization Buffer. Resuspend the reactions in 75 μ L of Reporter Mix by gently pipetting up and down several times. Proceed with step 16.