SAMPLE PROTOCOL FOR OLIGONUCLEOTIDE HYBRIDIZATION

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

1. Select appropriate oligonucleotide-coupled microsphere sets.

2. Resuspend microspheres by vortex and sonication for approximately 20 seconds.

3. Prepare a Working Microsphere Mixture by diluting coupled microsphere stocks to 150 microspheres of each set/μL in 1.5X TMAC Hybridization Solution. (Note: 33 μL of Working Microsphere Mixture is required for each reaction.)

4. Mix the Working Microsphere Mixture by vortex and sonication for approximately 20 seconds.

5. To each sample or background well, add 33 μL of Working Microsphere Mixture.

6. To each background well, add 17 μL TE, pH 8.

7. To each sample well, add biotinylated complementary oligonucleotide (5 to 200 femtomoles) and TE, pH 8.0 to a total volume of 17 μL.

8. Mix reaction wells gently by pipetting up and down several times.

9. Cover the reaction plate to prevent evaporation and incubate at 95-100°C for 1 to 3 minutes to denature any secondary structure in the sample oligonucleotides. *****

10. Incubate the reaction plate at hybridization temperature for 15 minutes. *****

11. Prepare fresh Reporter Mix by diluting streptavidin-R-phycoerythrin to 10μg/mL in 1X TMAC Hybridization Solution. (Note: 25 μL of Reporter Mix is required for each reaction.)

12. Add 25 μL of Reporter Mix to each well and mix gently by pipetting up and down several times.

13. Incubate the reaction plate at hybridization temperature for 5 minutes.

14. Analyze 50 μL **at hybridization temperature** on the Luminex analyzer according to the system manual.

***** ***These steps can be combined with the use of a thermal cycler programmed as follows –***
Hold at 95°C, 1 (to 3) minutes
Hold at hybridization temperature, FOREVER