

SAMPLE PROTOCOL FOR DIRECT DNA HYBRIDIZATION – WASHED ASSAY FORMAT USING MAGNETIC MICROSPHERES

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

1. Select the appropriate oligonucleotide-coupled microsphere sets.
2. Resuspend the 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 amplified biotinylated DNA and TE, pH 8.0 to a total volume of 17 μL . (Note: 2-5 μL of a robust PCR reaction is usually sufficient for detection.)
8. Mix reaction wells gently by pipetting up and down several times.
9. Cover the plate to prevent evaporation and incubate at 95-100°C for 5 minutes to denature the amplified biotinylated DNA. *
10. Incubate the plate at hybridization temperature for 15 minutes. *
11. Prepare fresh Reporter Mix by diluting streptavidin-R-phycoerythrin to 2-4 $\mu\text{g}/\text{mL}$ in 1X TMAC Hybridization Solution. (Note: 75 μL of Reporter Mix is required for each reaction.)
12. Place the plate into the magnetic separator and allow separation to occur for 30 to 60 seconds. See **Technical Note**.
13. Use a multi-channel pipette to aspirate the supernatant from each well. Take care not to disturb the microspheres.
14. Remove the plate from the magnetic separator and return the plate to hybridization temperature.
15. Resuspend the reactions in 75 μL of Reporter Mix by gently pipetting up and down several times.
16. Incubate the plate at hybridization temperature for 5 minutes.
17. 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, 5 minutes
Hold at hybridization temperature, FOREVER

Technical Note: For a list of magnetic separator plates, see **Recommended Materials for Magnetic Microspheres**. Optimal separation time may vary with the type of separator used.