HER2-006M Assay Procedure

A general procedure is as follows:

1. Before any experiment, it is necessary to wash the magnetic beads and remove the trehalose from the formulation buffer and change the buffer to your Assay/Washing Buffer. Immediate use is strongly recommended.

2. Add 1mL Assay/Washing Buffer per mg Beads to resuspend the beads. The most common Assay/Washing Buffer is PBS, pH 7.3, with 0.05% Tween-20. Optionally, you may add 0.05% BSA. When assaying a serum sample, please choose a Special Assay/Blocking Buffer to minimize the background signal.

3. Sample Dilution: Dilute your antibody of interest from 20μg/mL to 0.039μg/mL in Assay buffer.

4. Add 100 μL beads to each tube or plate well. Place the beads on the magnetic separator for 1-2 min. Remove the supernatant.

5. Add 100 μL diluted sample to the pelleted beads, and mix the beads with samples by mixer. Add 100 μL of assay buffer as a blank control into wells containing the beads.

6. Cover the tubes on a rotator or place the plate on a plate mixer and incubate for 60 minutes at room temperature. Alternatively, rotate overnight at 4°C.

7. Place the tube/plate on the magnetic separator for 2 min. Remove the supernatant.

8. Remove the tube/plate from the magnetic separator and resuspend the pelleted beads in 200 μL of Assay/Washing Buffer by a vortex.

9. Wash the beads for a total of 4 times by repeating steps 7–8. After the last wash, remove the supernatant.

10. Dilute your Secondary Antibody in Assay buffer. Add 100μL secondary antibody (at an appropriate dilution ratio) to the beads. We recommend PE anti-Human IgG Fc (Biolegend, Cat. No. 409304) at 1:200 to detect your human IgG antibody samples. Any other fluorescent-labeled secondary antibody is appropriate.

11. Place the tubes on a rotator or place the plate on a plate mixer, and incubate for 60 minutes at room temperature. Cover with foil to avoid photobleaching.

12. Repeat steps 7-8 for a total of four washes with Assay/Washing buffer. After the last wash remove the supernatant.

13. Add 100 μL of Assay/Washing Buffer to the beads, and resuspend the Beads.

14. Transfer the 100μL beads into a 96-well black plate that is compatible with your plate reader. Keep the beads well suspended.

15. Read the plate at excitation 488 nm/emission 575 nm on a plate reader within 10 min (Avoid the precipitation of the beads).