Linear Relationship between AFP and Lysine

Preparation of the beads:

1. Resuspend the in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Dynabeads to a tube.
3. Add an equal volume of Washing buffer, or at least 1 m L, and mix (vortex for 5 sec, or keep on a roller for at least 5 min).
4. Place the tube on a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed Dynabeads in the same volume of washing buffer as the initial volume of Dynabeads taken from the vial (step 2).

Immobilize Nucleic Acids:

1. Resuspend beads in 2X B&W Buffer to a final concentration of 5 μg/μL (twice original volume).
2. To immobilize, add an equal volume of the biotinylated AP273 in distilled water to dilute the NaCl concentration in the 2 B&W Buffer from 2M to 1M for optimal binding.
3. Incubate for 15 min at room temperature using gentle rotation.
4. Separate the biotinylated AP273 coated beads with a magnet for 2–3 min.
5. Wash 2–3 times with a 1X B&W Buffer.
6. Resuspend to the desired concentration. Binding is now complete, suitable for downstream applications.

Combination of Apt and Fluorescent Complementary Chain：

1. Take 10μl of the above-mentioned washed magnetic beads to add to 12 1.5 ml centrifuge tubes
2. Add 120μl of 1μmol/L of the fluorescent complementary strand and 40μl of 1μmol/L of AP273 to each of the centrifuge tubes
3. Heated the mixed solution from room temperature to 90℃and then cooled to room temperature. At this time, the fluorescent complementary strand is bonded to the aptamer by hydrogen bonding.
4. Place the mixed solution on the magnetic frame for 5min, discard the supernatant, and repeatedly wash on the magnetic frame to remove the unbound complementary 5. And then added the concentration of 2,4,6,8μg / ml of AFP solution to 12 centrifuge tubes .incubated for 2 hours at 37 ℃, place every tube in the magnetic frame for 5min, absorb 100μl of supernatant from every tube.
6. Measured the fluorescence value at an absorption wavelength of 492nm and an emission wavelength of 518nm.