The specific binding of AFP to AP-273

1. Coated: take 20μL (10mg / ml) Purified protein(AFP), then take 1980μL coated buffer, 100μL per well to add in 96-wells plate, Incubate in a 37 ℃ incubator for 2 hours.
2. Emptied and dry the residual liquid, and washed the 96-wells plate twice with 300μl of washing buffer.
3. Added 300μL (5 mg / ml) of BSA block Buffers to each well and allowed to stand at 4℃ for 1 hour
4. Discard the liquid, wash the plate with 300μl washing-buffer 3 times, during the last time after washing empty the liquid and pat dry
5. The aptamer was arranged into diluted solution of 0.2,0.4,0.6,0.8,1.0μmol/L in concentration, 100μl of the appropriate concentration of the aptamer was added to 96-wells .The blank group was added with TE buffer, and the above substances were incubated at 37 ℃for 2h.
6. Discard the liquid, wash the plate with 300μl washing-buffer 3 times, during the last time after washing empty the liquid and pat dry.
7. Added 100μl 1: 2000 diluted HRP- streptavidin to the 96-wellss and incubated at 37 ° C for 1.5 h.
8. discard the liquid，wash the plate with 300 μl of washing buffer three times, then soak with 300μl of rinse buffer for 5 min, then empty the liquid and pat dry，and then wash the plate with 300μl washing buffer 2 times，during the last time after washing empty the liquid and pat dry.
9. Added 10μL of TMB solution to 96-wells. After 20 mins of color development in the dark, added 50μL stop solution to terminate the reaction immediately. After the reaction was stopped, read the absorbance value at 450 nm.

EDC Plus Sulfo-NHS

1. Dissolve 1mg L-lysine in 1ml of activation buffer (0.1M MES, 0.5M NaCl,

pH6.0), at a concentration of 1mg/ml.

2. Add coupled reagent EDC 0.4mg and sulfo-NHS 0.6mg to the solution in step 1, at a concentration of 0,4mg/ml of EDC and 0.6mg/ml of sulfo-NHS.
3. Shake up and react for 15min in dark at room temperature.
4. Add 1.4ul β-Mercaptoethanol to the reaction solution. Mix and incubate for 10min at room temperature to neutralize extra EDC.
5. Add 10ul amino-modified complementary strand(100uM) to coupling buffer(phosphate buffer 100mM sodium phosphate,150mM NaCl, pH 7.2)
6. Adjust the pH of the activation buffer over 7.0.
7. Mix the activation buffer with the coupling buffer, shake up and react for 2 hours in dark at room temperature.
8. Use ultrafiltration to concentrate and purify the solution after the end of the reaction.9
9. Using mass spectrometer and infrared detector for testing.

The Deprotection of Boc-Lysine

In this part we choose TEA (Trifluoroacetate) to realize the deprotection of Boc-Lysine.

1. Add Tert-Butoxycarbonylaminocaproic acid (boc-lys, 4g) to a mixed solution of trifluoroacetic acid (5ml) and dichloromethane (10ml).
2. Stir the solution for 1 hour under a room temperature till the reaction was terminated.
3. Evaporate impurities under rotation conditions.
4. Dissolve the remaining material in an appropriate amount of ethyl acetate (5 ml), washed with 5% Na_2CO_3 (25-30 ml) solution to pH 8-9.
5. Remove the solvent by rotary evaporation to get the lysine.

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.