Detection Antibody HRP labeling
For diagnostics application (ELISA)

1. Dissolve 5mg HRP in 1ml distilled water.

2. Add 200 μL of 0.1M NaIO4 solution [1] (freshly prepared just before use) to the solution obtained in step 1 and stirred for 20 minutes at room temperature. Protect from light.

3. Put the solution obtained in step 2 into a dialysis bag and dialyzed with 1mM sodium acetate buffer [2] (PH4.4) at 4°C overnight.

4. Dissolve 10mg antibody in 1mL 0.01M carbonate buffer.

5. Add 20 μL 0.2M PH 9.5 carbonate buffer [3] to adjust the PH of the solution obtained in step 3 to 9.0~9.5, then immediately add to the antibody solution obtained in step 4, and gently stir at room temperature for 2 hours. Protect from light.

6. Add 100 μL 4mg/ml NaBH4 solution [4] (freshly prepared just before use), mix well, 4°C for 2 hours.

7. Filter the reaction mixture obtained in step 6 was by Sephadex-25 column, elute with PBS, and determine the optical density at 280 nm and 403 nm of each well.

8. Collect the solutions of the wells with light absorption both at 280 nm and 403 nm, which is HRP-labeled antibody conjugates, use directly or store at -20 °C.

[1] 0.1M NaIO4 solution:

NaIO4 241 mg

Add distilled water to 10ml

[2] 1mM PH4.4 sodium acetate buffer:

0.2M NaAc 3.7ml

0.2M HAc 6.3ml

Add distilled water to 2000ml

[3] 0.2M PH 9.5 carbonate buffer:

Na2CO3 0.32g

NaHCO3 0.586g

Add distilled water to 50ml

[4] 4mg/ml NaBH4 solution:

NaBH4 4mg

Add distilled water to 1ml

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