Protocol For BioPair Antibody-Coupling MagBeads

Equipment

- Ice bath
- Micropipettor
- Micropipetting tips
- Glass bottles (500 ml, 1000 ml)
- pH meter
- Vortex mixer
- End-over-end shaker

Materials

- Sodium chloride (NaCl)
- Disodium hydrogen phosphate dihydrate (Na2HPO4 x 2H2O)
- Sodium carbonate monohydrate (Na2CO3 x H2O)
- Sodium acetate trihydrate CH3COONa x 3H2O)
- Acetic acid, 100% (CH3COOH)
- Ethanol, absolute (C2H5OH)
- Sodium hydroxide (NaOH)

Solutions and Buffers

Phosphate Buffer, 100 ml (100 mM Na phosphate, 150 mM NaCl, pH 7.2)

Instructions: 8.72 g sodium chloride and 17.8 g disodium hydrogen phosphate dihydrate are added to 900 ml deionized water and the pH is adjusted to 7.2. Subsequently, this should be made up to 1000 ml.

Storage Buffer

Instructions: 375 ml Millipore water, 1.345 g sodium acetate trihydrate and 7 µl acetic acid are added to a 500 ml glass bottle. Then, if necessary, adjust the pH to 6.50 - 6.60 and add 125 ml abs. ethanol.

Activation Buffer

Instructions: Stock solution (800 µl of 37% hydrocloric acid solution in 100 ml dd-water). Dissolute 10 ml of stock solution in 1 l of dd-water.

Procedure

1. Prepare a solution of up to 100 µg antibody or other biomolecule in 500 µl Phosphate Buffer or PBS. Make sure that no primary amines (Tris, ethanolamine, etc.) are present in the Buffer.
2. Wash with 500 µl of Activation Buffer. Mix well and incubate for 2 minutes, remove the supernatant by magnetic separation. Repeat step 2.
3. Add the antibody solution to the magnetic particles. Mix by swirling or gently vortexing.
4. Transfer the test tube to a suitable device for mixing (e.g., roller, end-over-end shaker). Allow the reaction mixture to incubate for 16 hours at 4 °C. Make sure that the sample is mixed well.
5. After the reaction time, transfer the reaction tube to a suitable magnetic separator. Remove the supernatant.
6. Add 500 µl of Phosphate Buffer or PBS. Mix, separate magnetically and discard the supernatant.
7. Repeat the Phosphate or PBS Wash two times.
8. Take up the magnetic particles in Storage Buffer. Adding 200µl of Storage Buffer to the 50 mg Magbeads equals a standard suspension of 25 %. The solution should be stored at 4 °C and is thus stable for up to two years, depending on the antibody or biomolecule.

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