Covalent Coupling Procedure of Sulfhydryl Group Containing Ligands on Bromoacetyl Magnetic Particles

Equipment and Buffer

Bromoacetyl Magnetic Particles
Coupling Buffer: 50 mM Tris, 5 mM EDTA-Na, pH 8.5
Blocking Buffer: 50 mM L-Cysteineÿ-HCl in Coupling Buffer
Storage Buffer: PBS, 0.05 % sodium azide
Magnetic Separator

Protocol

The following protocol describes the coupling of biomolecules on 10 mg particles. The procedure can be scaled up by adjusting volumes of required reagents.

1. Wash the Bromoacetyl Magnetic Particles 2 x with 1 ml Coupling Buffer using a magnetic separator and resuspend the particles in 0.25 ml Coupling Buffer by vortexing.
2. Add the sulfhydryl group containing ligands to the particles and mix the suspension on a shaker for 15 minutes at room temperature.

Note: Dissolve the sulfhydryl group containing ligands with Coupling Buffer. If the sample is not soluble in Coupling Buffer, dissolve it in a suitable buffer at pH 8-8.5. Dilute samples already in solution 1:1 in Coupling Buffer.

3. Wash the particles 2 x with 1 ml Coupling Buffer.
4. Add 0.5 ml Blocking Buffer to the particles and mix the suspension on a shaker for 15 minutes at room temperature.
5. Separate the magnetic particles by using a magnetic separator, discard the supernatant and resuspend the particles in an appropriate volume of Storage Buffer.

Troubleshooting

Problem	Answer
Sample ligands preciptates in Coupling Buffer Ligands are not soluble in Coupling Buffer.	Dissolve sample in 30% Dimethylsulfoxid or Dimethylformamid or 6 M guanidineÿ-HCl.
Low coupling efficiency Sulfhydryl groups not reduced.	Reduce the ligands and proceed immediately with desalting and coupling procedure to prevent reformation of disulfide bonds.

This protocol is intended to provide general guidelines for the binding of biomolecules or related compounds. Further optimization may be required in order to achieve optimal functionality and stability from case to case.

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