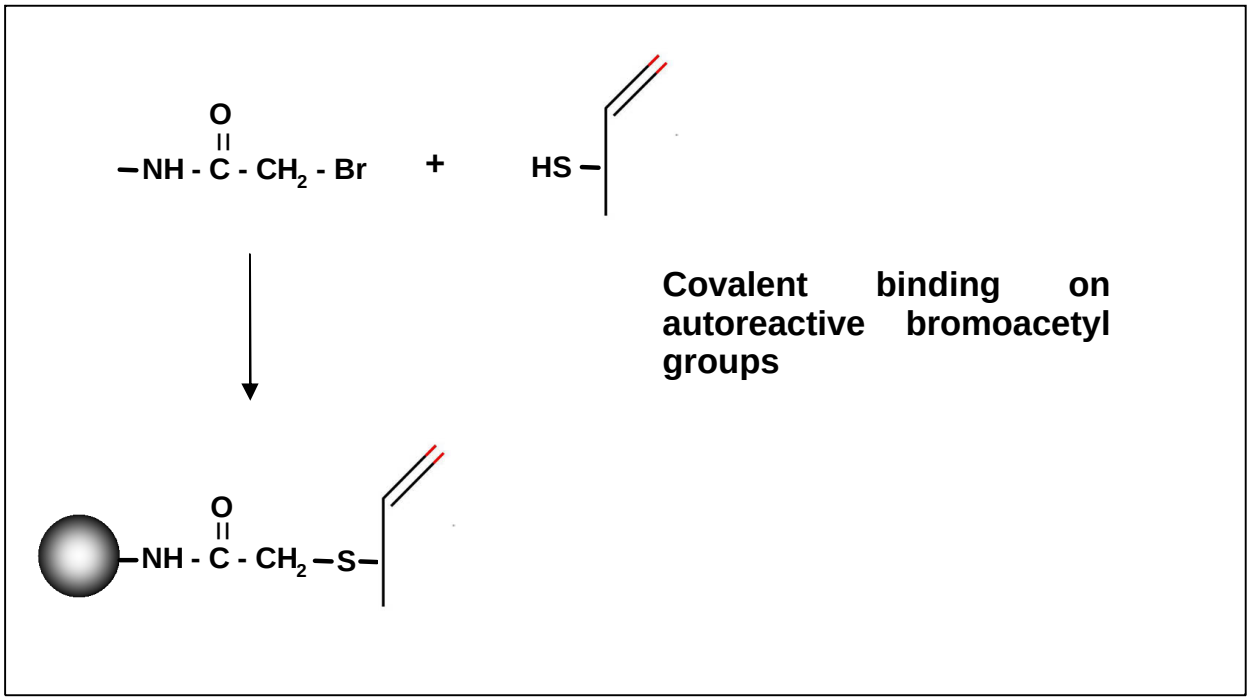
Covalent Coupling Procedure of sulfhydryl group containing ligands on Bromoacetyl Magnetic Particles, Silica

Introduction:

This procedure describes covalent coupling of sulfhydryl groups containing ligands such as antibodies, proteins or low molecular substances to autoreactive Bromoacetyl Magnetic Particles, Silica with very high efficiency without further activation.

The coupling reaction with sulfhydryl groups containing proteins is very fast (30 min.) and the coupling product offers extremely stable thioether bonds between Bromoacetyl Magnetic Particles, Silica and the ligand.



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Equipment and reagents:

- **Bromoacetyl Magnetic Particles, Silica**
- **Coupling Buffer:** 50 mM Tris, 5 mM EDTA-Na, pH 8.5
- **Blocking Buffer:** 50 mM L-Cysteine - HCl in Coupling Buffer
- **Storage Buffer:** PBS, 0.05 % sodium azide
- **Magnetic Separator** (**e.g.** CD Iso™ 1.5 mL Magnetic Tube Separator**,** Cat.#: WHK-MS007)

Technical Note:

- We recommend to use a minimum amount of 50 µg sulfhydryl containing ligands per 10 mg Bromoacetyl Magnetic Particles, Silica. In general, the higher the amount of sulfhydryl containing ligands per milligram of Bromoacetyl Magnetic Particles, Silica, the higher will be the degree of magnetic particle surface coating with the ligands.
- **Store the beads at 4°C protected from light. Alkyl halide-containing compounds are extremely light sensitive.**

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, Silica 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.

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Protocol:

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.

Trouble shooting:

Problem	Answer
Sample ligands preciptates in Coupling Buffer ▪ Ligands are not soluble in Coupling Buffer.	▪ Dissolve sample in ≤ 30% *DMSO or **DMF or 6 M guanidine - 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.

*DMSO (**Dim**ethyl**s**ulf**o**xid); **DMF (**Dim**ethyl**f**ormamid)

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