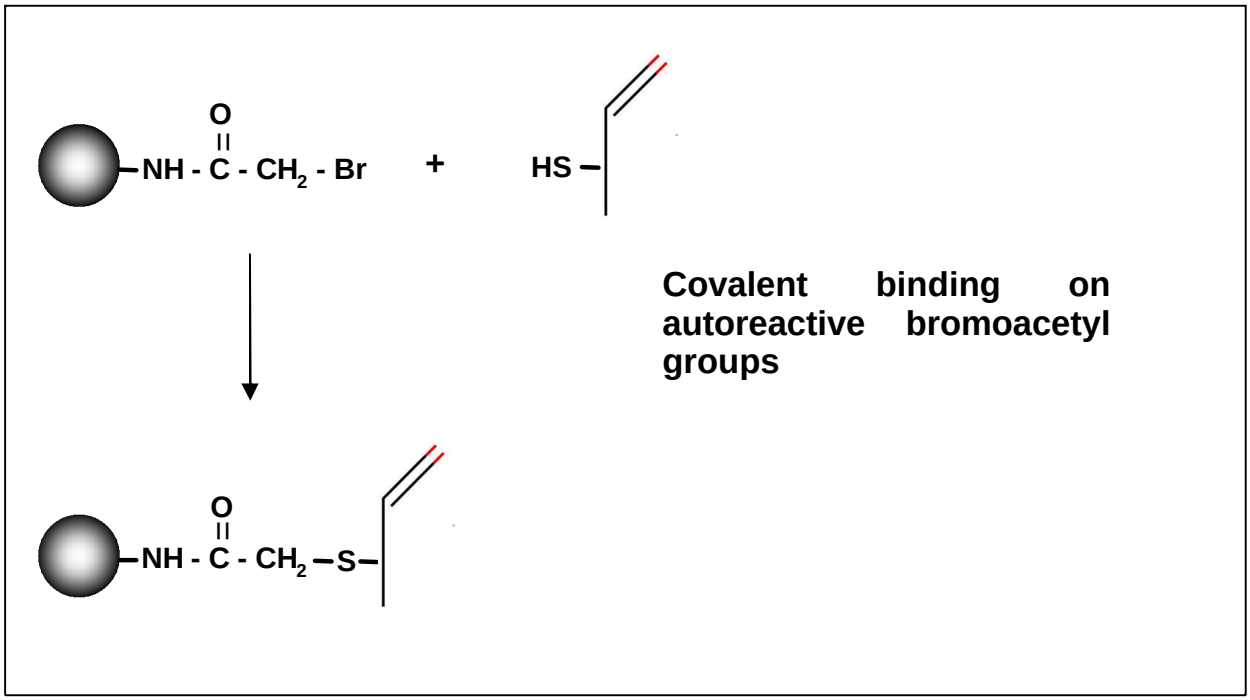
Covalent Coupling Procedure of sulfhydryl group containing ligands on SiMAG-Bromoacetyl and fluidMAG-Bromoacteyl

Introduction:

This procedure describes covalent coupling of sulfhydryl groups containing ligands such as antibodies, proteins or low molecular substances to autoreactive **SiMAG-Bromoacetyl / fluidMAG-Bromoacetyl** with very high efficiency without futher activation.

The coupling reaction with sulfhydryl groups containing proteins is very fast (30 min.) and the coupling product offers extremely stable thioether bonds between **SiMAG-Bromoacetyl / fluidMAG-Bromoacteyl** and the ligand.



Equipment and reagents:

- **SiMAG-Bromoacetyl / fluidMAG-Bromoacetyl**
- **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. MagnetoPURE,** Product Number: MP-10)

Technical Note:

- We recommend to use a minimum amount of 50 µg sulfhydryl containing ligands per 10 mg **SiMAG-Bromoacetyl / fluidMAG-Bromoacetyl**. In general, the higher the amount of sulfhydryl containing ligands per milligram of **SiMAG-Bromoacetyl / fluidMAG-Bromoacetyl**, 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 **SiMAG-Bromoacetyl / fluidMAG-Bromoacetyl** 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.

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.

Troubleshooting:

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)