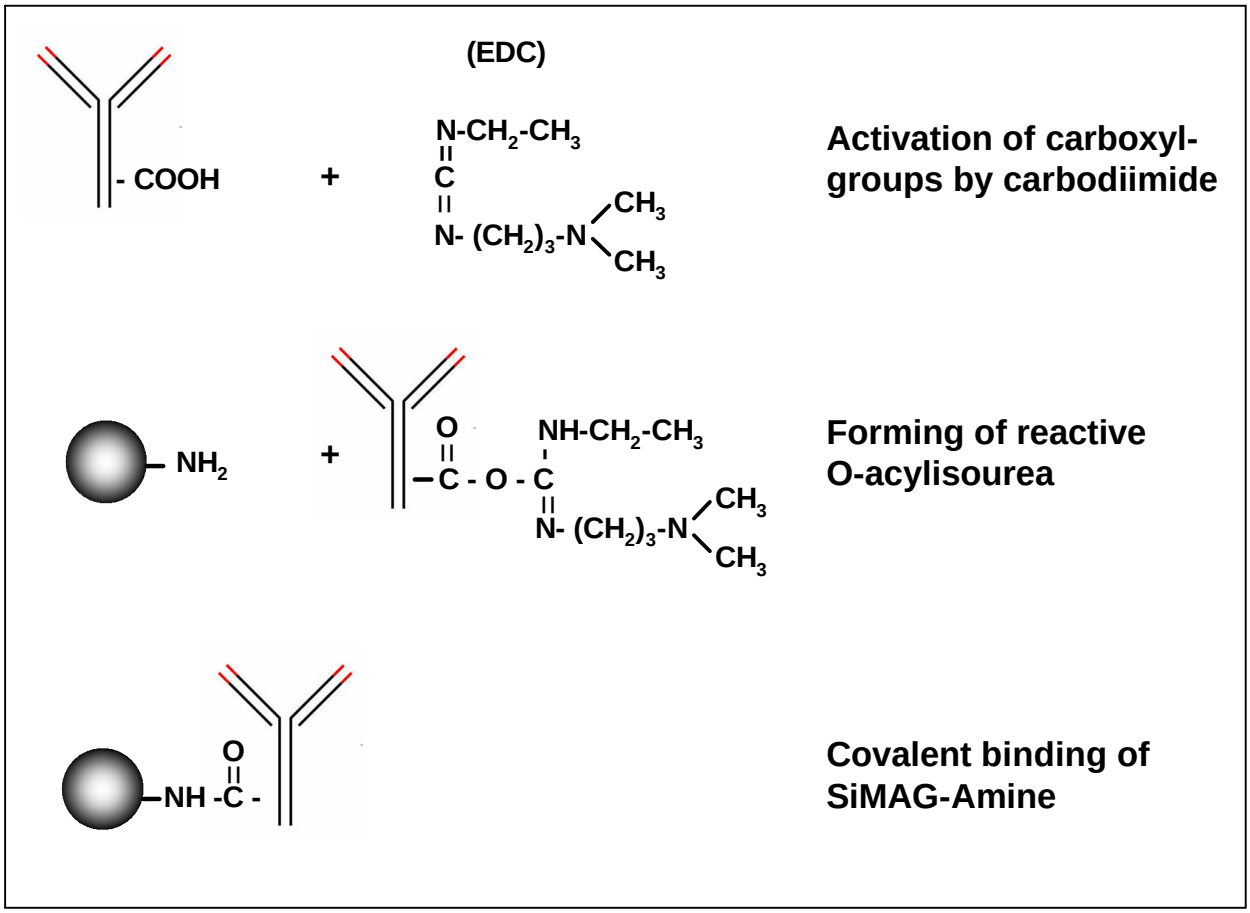
Covalent Coupling Procedure on SiMAG-Amine by Carbodiimide Method

Introduction:

This procedure describes covalent coupling of carboxyl-group containing ligands such as antibodies, proteins or low molecular substances to **SiMAG-Amine** by the carbodiimide method.

The carbodiimide method is a binary covalent binding system and guarantees therefore a good reproducibility of the immobilization.

Carbodiimides react with the terminal amine-groups from the magnetic beads to highly reactive O-acylisourea derivatives and react readily with carboxyl-groups of the ligands.



Equipment and reagents:

- **SiMAG-Amine**
- **Wash & Coupling Buffer:**

  0.1 M 2-(N-Morpholino)ethanesulfonic acid (MES), pH 6.0

- **Water Soluble Carbodiimide:**

  1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)
  or
  1-cyclohexyl-3(2-morpholinoethyl) carbodiimide metho-p toluensulfonate (CMC)

- **Blocking & Storage Buffer:**

  PBS, 0.1 % BSA, 0.05 % sodium azide

- **Magnetic Separator** (**e.g. MagnetoPURE,** Product Number: MP-10)

Technical Note:

- For an optimal binding capacity of the molecule of interest it is possible to optimize the pH value between pH 5.5 - 6.5 of the Washing & Binding Buffer.
- All buffers used for activation or coupling may not contain carboxyl-groups, proteins or high salt conditions.
- For antibodies or proteins, we recommend to use a minimum amount of 50 µg antibody/protein per 10 mg **SiMAG-Amine**. In general, the higher the amount of antibody/protein per milligram of **SiMAG-Amine**, the higher will be the degree of magnetic particle surface coating with the protein.
- **Prepare the EDC solution immediately before use and mix the volume rapidly into the reaction tube.**

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

Protocol:

1. Wash the **SiMAG-Amine** particles 2 x with 1 ml MES buffer using the magnetic separator.

2. After the second wash step resuspend the magnetic particles in 0.25 ml MES buffer containing 10 mg EDC or CMC. Add only **freshly prepared EDC** to the particles and mix at room temperature.

3. Add carboxyl group containing ligands (e.g. 50 µg protein dissolved in ddH_2O) to the particles and mix the suspension on a shaker for 2 hours at room temperature.

4. Wash the particles 3 x with 1 ml PBS.

5. Resuspend the particles in Blocking/Storage buffer.