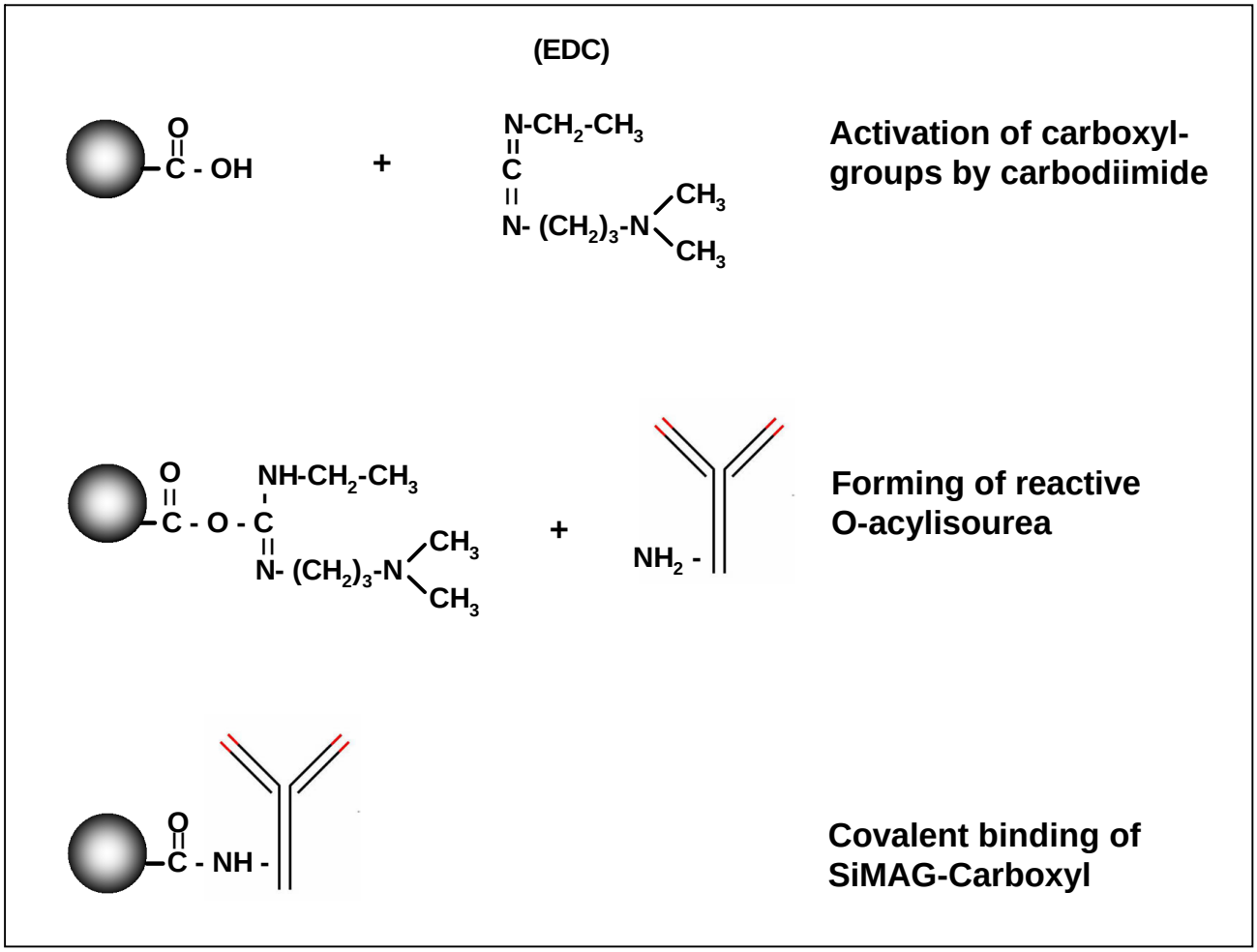
Covalent Coupling Procedure on SiMAG-Carboxyl by Carbodiimide Method

Introduction:

This procedure describes covalent coupling of amino- group containing ligands such as antibodies, proteins or low molecular substances to **SiMAG-Carboxyl** 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 carboxylate-groups from the magnetic beads to highly reactive O-acylisourea derivatives and react readily with amino-groups of the ligands.



Equipment and reagents:

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

  0.1 M 2-(N-Morpholino)ethanesulfonic acid (MES), pH 5.0

- **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:

- We recommend for high molecular ligands, such as antibodies or proteins, the 2-step method for the prevent of cross linking effects. The 1-step method without washing after the EDC addition (2.) is more effective for the coupling of low molecular ligands.
- For an optimal binding capacity of the molecules of interest it is possible to optimize the pH value between pH 4.0 - 6.5 of the Washing & Binding Buffer.
- All buffers used for activation or coupling may not contain amino-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-Carboxyl. In general, the higher the amount of antibody/protein per milligram of SiMAG-Carboxyl, 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-Step Method:

1. Wash the **SiMAG-Carboxyl** 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 on a shaker for 10 minutes at room temperature.
3. Add amine group containing ligands (e.g. 50 µg protein dissolved in ddH_2O) to the activated 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.

2-Step Method:

1. Wash the **SiMAG-Carboxyl** 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 on a shaker for 10 minutes at room temperature.
3. After incubation wash the particles 2 x with 1 ml MES buffer and resuspend the activated particles in 0.25 ml MES buffer.
4. Add amine group containing ligands (e.g. 50 µg protein dissolved in ddH_2O) to the activated particles and mix the suspension on a shaker for 2 hours at room temperature.
5. Wash the particles 3 x with 1 ml PBS.
6. Resuspend the particles in Blocking/Storage buffer.