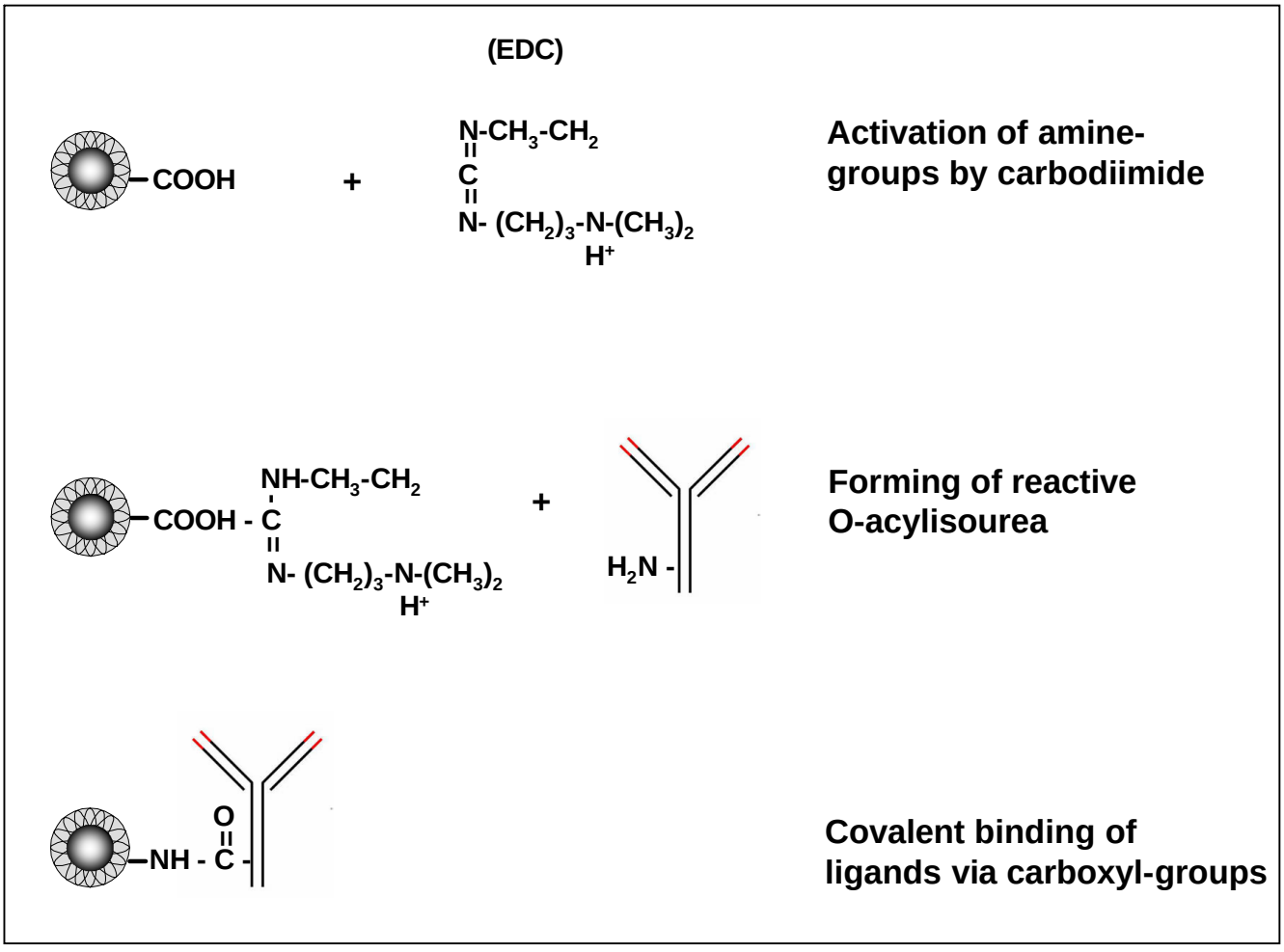
Covalent Coupling Procedure on fluidMAG-CMX by Carbodiimide Method

Introduction:

The coupling procedure with carbodiimides are a binary covalent binding system and guarantee therefore a good reproducibility of the immobilization.

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



Equipment and Reagents:

- **fluidMAG-CMX**
- **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 recommended 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 is more effective for the coupling of low molecular ligands.
- For antibodies or proteins we recommend to use a minimum amount of 50 µg antibody/protein per 10 mg fluidMAG-CMX. In general, the higher the amount of antibody/protein per milligram of fluidMAG-CMX, 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.

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

Protocol:

One-Step Method

1. Add 10 mg **fluidMAG-CMX** particles in a fresh clean microtube.

2. Dissolve 10 mg **EDC** or **CMC** in 0.15 ml ddH_2O. Add **freshly prepared EDC** to the particles and mix gently.

3. Add proteins (e.g. 50 µg protein dissolved in PBS) to the activated particles and mix the suspension gently for two hours at room temperature.

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

5. Resuspend the particles in **Blocking & Storage Buffer**.

Two-Step Method

1. Add 10 mg **fluidMAG-CMX** particles in a fresh clean microtube.

2. Dissolve 10 mg **EDC** or **CMC** in 0.15 ml ddH_2O. Add **freshly prepared EDC** to the particles and mix gently for 10 minutes at room temperature.

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3. After incubation wash the particles 2 x with 1 ml ddH_2O and resuspend the activated particles in 0.25 ml ddH_2O.

4. Add proteins (e.g. 50 µg protein dissolved in PBS) to the activated particles and mix the suspension gently for two hours at room temperature.

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

6. Resuspend the particles in **Blocking & Storage Buffer**.

Troubleshooting:

Problem	Answer
Low coupling efficiency ▪ Primary amines not completely removed from sample before coupling	▪ Completely remove primary amines by extensive dialysis or desalting
Clumping after / during procedure • Carbodiimide addition causes clumping • EDC addition causes clumping • Protein addition causes clumping	• Decrease particle concentration • Decrease EDC concentration • Decrease protein concentration