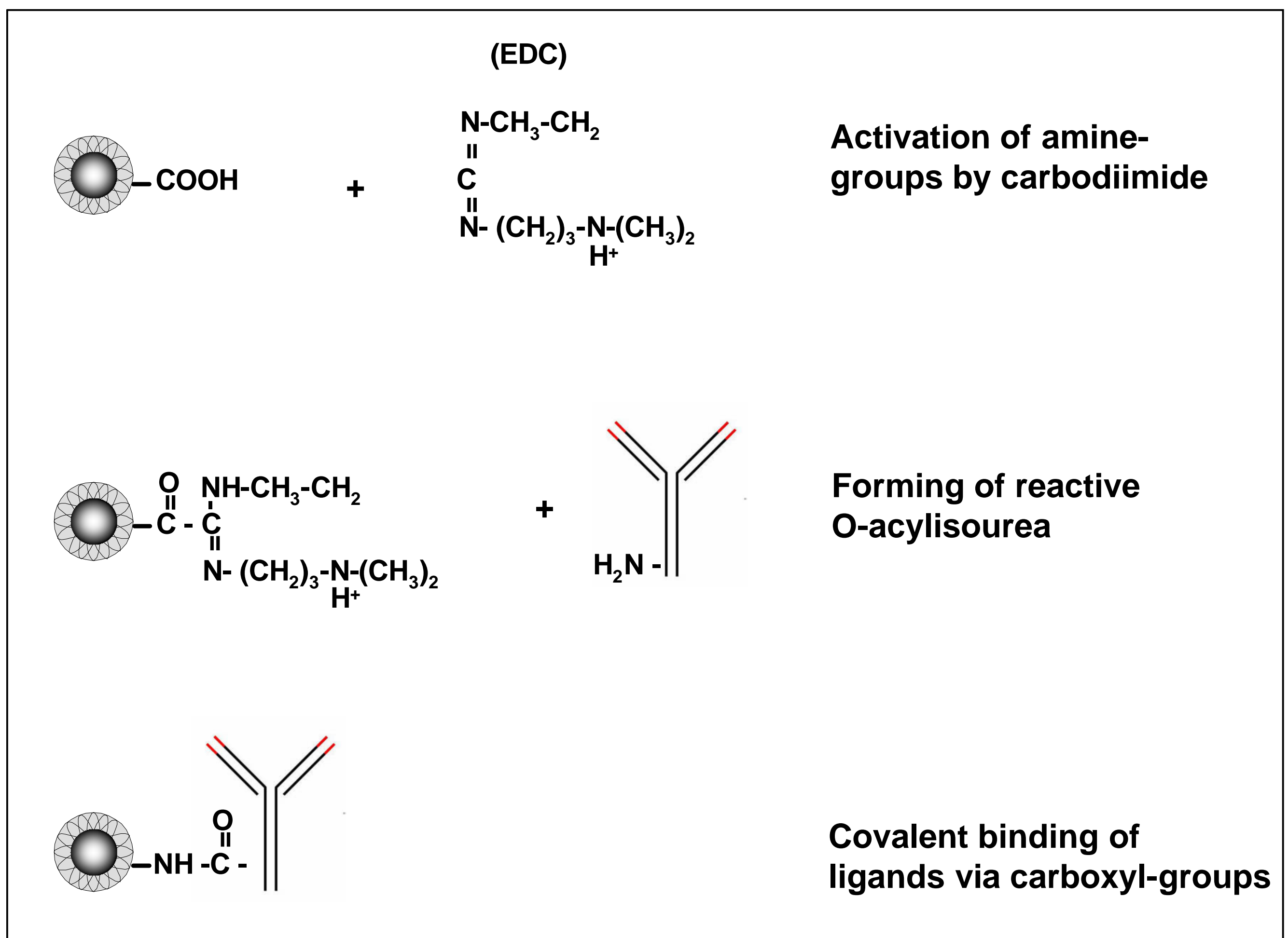
Covalent Coupling Procedure on fluidMAG-ARA by Carbodiimide Method

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-ARA**

• **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 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 (3.) 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-ARA**. In general, the higher the amount of antibody/protein per milligram of **fluidMAG-ARA**, 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:

1. Wash the **fluidMAG-ARA** particles 2 x with 1 ml MES buffer by using of the magnetic separator.

2. After the second wash step resuspend the magnetic particles in 0.25 ml MES buffer.

3. Dissolve 10 mg EDC or CMC in 0.25 ml MES buffer. Add **freshly prepared EDC** to the particles and mix gently for 10 minutes at room temperatur.

2-step methode

4. After incubation wash the particles 2 x with 1 ml MES buffer and resuspend the activated particles in 0.25 ml MES buffer.

1-step and 2-step procedure

5. Add proteins (e.g.50 µg protein dissolved in MES buffer) to the activated particles and mix the suspension gently for two hours at room temperatur.

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

7. Resuspend the particles in Blocking/Storage buffer.