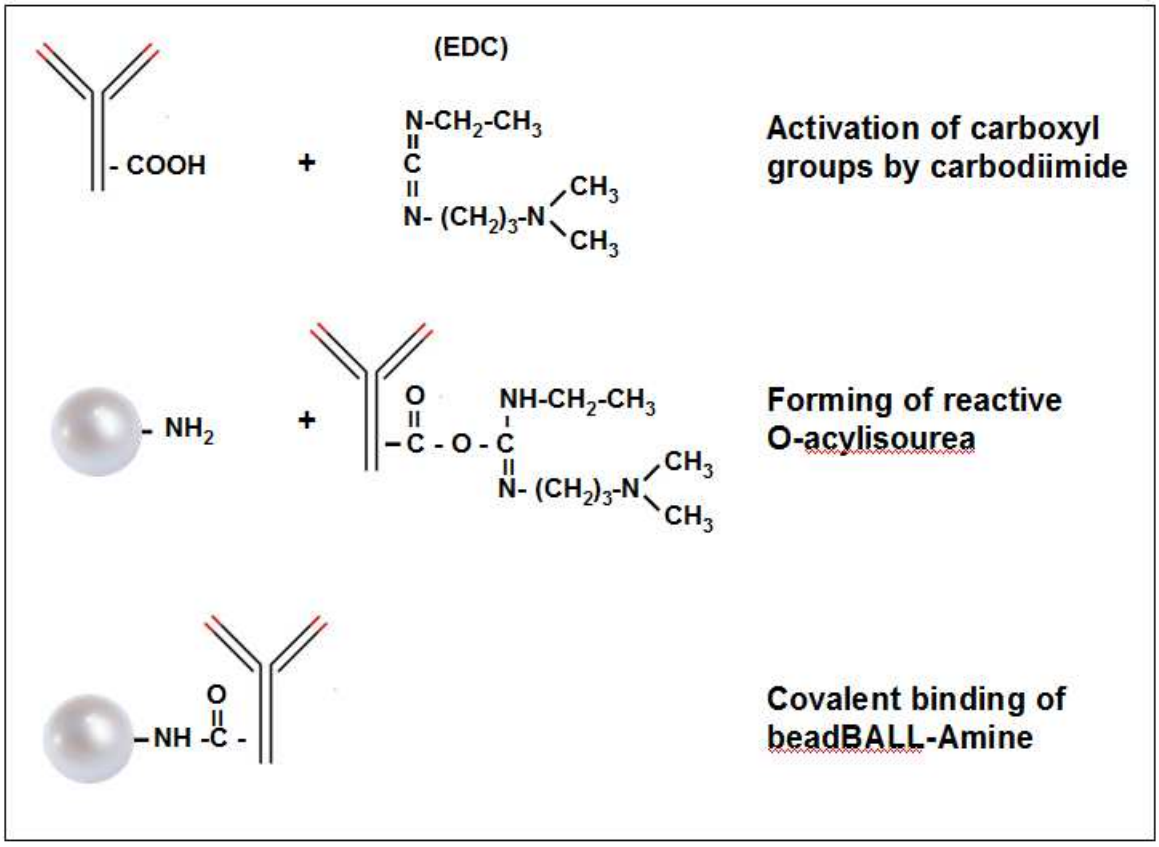
Covalent Coupling Procedure on beadBALL-Amine via Carbodiimide Method

Introduction:

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

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

Carbodiimides react readily with the terminal carboxylate groups of the ligand to highly reactive O-acylisourea derivatives, which then react readily with amino groups of the microspheres.



Equipment and reagents:

- **beadBALL-Amine**

- **MES 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 toluenesulfonate (CMC)

- **Blocking & Storage buffer:**
  PBS, 0.1 % BSA, 0.05 % sodium azide

- **Microcentrifuge**

Technical note:

- Regarding an optimal binding capacity of the target molecule, it is possible to adjust the pH value of the **MES buffer** between pH 5.5 - 6.5.

- All buffers used for activation or coupling may not contain any molecules with primary or secondary amino groups. Also a high salt condition should be avoided.

- Regarding the coupling of antibodies or proteins, we recommend to use a minimum amount of 50 µg antibody / protein per 10 mg **beadBALL-Amine**. In general, the higher the amount of antibody/protein per milligram of **beadBALL-Amine**, the higher will be the degree of coupled antibody / protein on the microsphere surface.

- **Prepare the EDC solution immediately before use and transfer the needed volume rapidly into the reaction tube.**

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

Protocol:

1. Transfer 10 mg **beadBALL-Amine** microspheres in a 2 ml microcentrifuge tube, add 1 ml **MES buffer** and centrifuge for 1 minute at 500 x g. Remove the supernatant, add 1 ml **MES buffer**, resuspend the pellet completely by thoroughly vortexing, centrifuge and remove the supernatant.

2. Add 0.25 ml **MES buffer** containing 10 mg EDC or CMC to the microspheres. Use only **freshly prepared EDC** solution. Mix on a shaker for 1 minute at room temperature.

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

4. Wash the particles 3 x with 1 ml PBS as described in position 1.

5. Resuspend the microspheres in an appropriate volume of **Blocking & Storage buffer.**