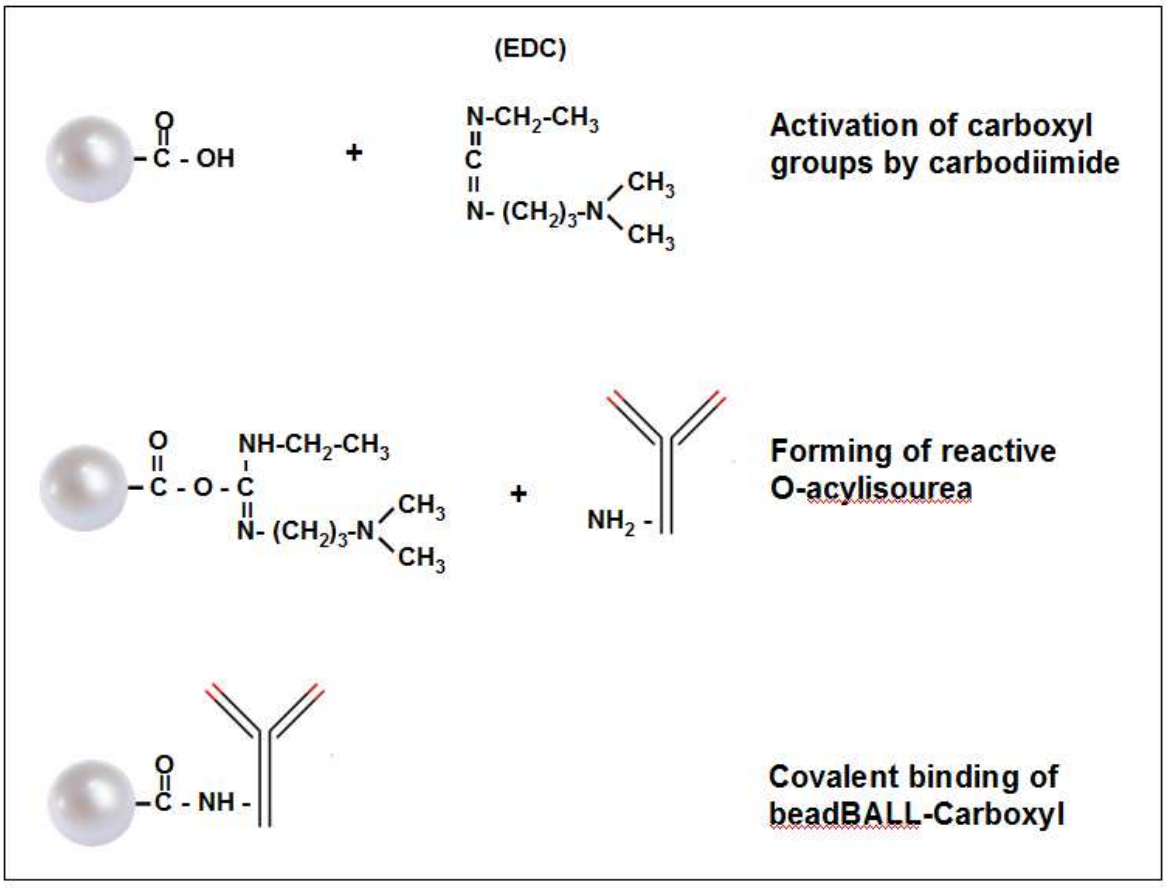
# Covalent Coupling Procedure on beadBALL-Carboxyl via Carbodiimide Method

## Introduction:

This procedure describes the covalent coupling of amino group containing ligands, such as antibodies, proteins or low molecular substances to **beadBALL-Carboxyl** 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 microspheres to highly reactive O-acylisourea derivatives, which then react readily with amino groups of the ligands.



## Equipment and reagents:

- **beadBALL-Carboxyl**

- **MES 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-toluenesulfonate (CMC)

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

- **Microcentrifuge**

## Technical note:

- Concerning high molecular ligands, such as antibodies or proteins, it is recommended to use the 2-step method to prevent cross linking effects. The 1-step method, without washing after EDC addition (2.), is more effective for the coupling of low molecular ligands.

- To optimize the binding capacity of the target molecule, it is possible to adjust the pH value of the **MES buffer** between pH 4.0 - 6.5.

- All buffers used for activation or coupling may **not** contain 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-Carboxyl**. In general, the higher the amount of antibody/protein per milligram of **beadBALL-Carboxyl**, the higher will be the degree of coupled antibody / protein on the microsphere surface.

- **Prepare the EDC solution immediately before use and mix transfer needed the 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-Step Method:

1. Transfer 10 mg **beadBALL-Carboxyl** 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 10 minutes at room temperature.

3. Add amine group containing ligands (e.g. 50 µg protein dissolved in $ddH_2O$) to the activated microspheres 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.**

### 2-Step Method:

1. Transfer 10 mg **beadBALL-Carboxyl** 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 10 minutes at room temperature.

3. Wash the microspheres 2 x with 1 ml **MES buffer** as described in position 1. and resuspend the activated microspheres in 0.25 ml **MES buffer**.

4. Add amine group containing ligands (e.g. 50 µg protein dissolved in $ddH_2O$) and mix the suspension on a shaker for two hours at room temperature.

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

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