

## Experiment Protocol 004

### Immobilization of ligands (carboxylic compounds) on OH beads

For screening, you need, first of all, to optimize the amount of immobilization of ligands on beads. You can change the amount of immobilization of ligands by changing the concentration of ligands. This experiment protocol shows a method to immobilize ligands at four various concentrations, i.e. 0 mM, 2 mM, 10 mM, and 50 mM when immobilizing ligands on OH beads.

#### 1. Materials

##### 1.1 Beads and Ligands (Compounds)

- Epoxy beads (TAS8848N1110): 10 mg (Functional groups : Approx. 200 nmol/mg)
- Ligands: Approx. 20 mg

##### 1.2 Reagents

- N,N-Dimethylformamide (DMF) 20 mL
- 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC· HCl) M.W. 191.70  
30 mg
- Triethylamine 200  $\mu$ L
- Methanol 4 mL

##### 1.3 Apparatus

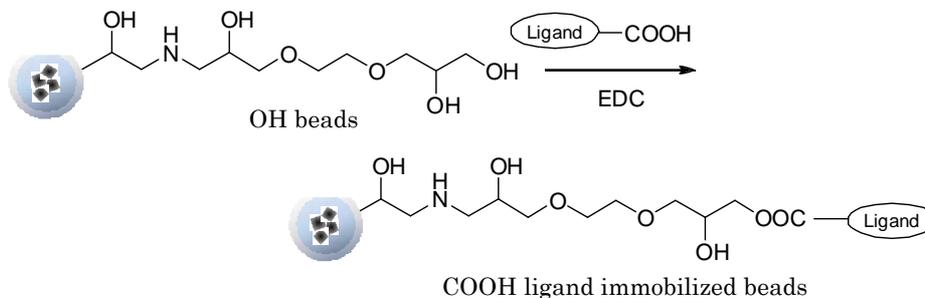
- Micro centrifuge
- Micro tube mixer (TOMY MT-360, etc.)
- Ultrasonic dispersing device

We have performed operation checks with an ultrasonic homogenizer: VP-15S with a cup horn (TAITEC), or an ultrasonic dispersing device: TA4905 (Tamagawa Seiki).

#### 2. Method

##### 2.1 Outline

The following is a schematic view of ligand immobilization. Refer to the next section 2.2 "Procedures" for details.



##### 2.2 Procedures

- 1) Dissolve ligands (compounds) in DMF, and prepare 500 $\mu$ L of 100 mM ligand solution.
- 2) Add 2.5 mg of OH beads (TAS8848N1120) into each of four 1.5 mL micro-tubes.
- 3) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.
- 4) Add 500  $\mu$ L of DMF, and disperse the beads with an ultrasonic device.
- 5) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.
- 6) Repeat the above 4) to 5) two more times. (Wash the beads three times in total.)
- 7) Add DMF, triethylamine and the prepared 100 mM ligand solution, and disperse the beads with an ultrasonic device. (Refer to the table on the next page.)
- 8) Add EDC·HCl of the five times molar quantity of ligands (e.g. 24 mg of EDC· HCl to 50mM of ligands), and mix them. (Or put the measured EDC·HCl into another tube, and add ligands and beads solution into the tube)

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Concentration	(mM)	0	2	10	50
OH beads	(mg)	2.5	2.5	2.5	2.5
DMF	( $\mu$ L)	450	440	400	200
100 mM ligand	( $\mu$ L)	0	10	50	250
Triethylamine	( $\mu$ L)	50	50	50	50
EDC· HCl	(mg)	0	1	5	24
Total	( $\mu$ L)	500	500	500	500

- 9) React for 16 to 20 hours (overnight) at room temperature by using a micro tube mixer.
- 10) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.
- 11) Add 500  $\mu$ L of DMF, and disperse the beads with an ultrasonic dispersing device.
- 12) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.
- 13) Repeat the above 11) to 12) two more times. (Wash the beads three times in total.)
- 14) Add 500 $\mu$ L of 50% MeOH, and disperse the beads with an ultrasonic device.
- 15) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.
- 16) Repeat the above 14) to 15) two more times. (Wash the beads three times in total.)
- 17) Disperse the beads in 100  $\mu$ L of 50% MeOH, and store at 4°C. (Concentration of ligand immobilized beads:0.5 mg/20  $\mu$ L)

### 3. Supplements

- It is more effective for the immobilization of carboxylic compounds to use NH<sub>2</sub> beads (TAS8848N1130) than to use OH beads because the OH beads have low reactivity, and the subsequent ester bond has low stability.
- Beads are easily dispersed by using an ultrasonic dispersing device. But if you do not have such a device, they are dispersed by using an ultrasonic washer, or by the manual agitation. In the manual dispersion method, the bottom of a micro-tube is glided over an uneven surface (side of plastic test tube rack in this case) creating turbulence through the collisions. (see left side picture below)  
Please make sure to use well-constructed tubes with the caps tightly secured in order to prevent leakage/breakage. Use of cap lock is recommended in order to prevent leakage. (see right side picture below).  
For more information, please visit FG beads web site and see the movie of the method.  
(Please click : <http://www.magneticnanoparticle.jp/en/htdocs/technique/affinity.html> for moving pictures.)



- Recover beads dispersed in DMF or 50% MeOH not by magnetic separation but by centrifugation because the magnetic separation takes a longer time.
- Use DMF which is hydrated with a molecular sieve, or a low-moisture solvent. If the solvent contains moisture, succinimide may be liberated from beads, and ligands are not properly immobilized on the beads.

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- Although we recommend using 50% MeOH for storing ligand immobilized beads in view of the decrease of dispersibility of beads due to immobilization of hydrophobic compounds, you can satisfactorily use ultrapure water, too.

### 4. Notes

- Because EDC· HCl are prone to absorb moisture, be careful not to contaminate them with moisture.
- You cannot store the ligand immobilized beads for a long time because binding of the ligands with the beads is an ester bond, and are susceptible to hydrolysis. Use the ligand immobilized beads as soon as possible after the immobilization.