

## Experiment Protocol 003

### Immobilization of ligands (compounds with phenol groups or NH<sub>2</sub> groups) on epoxy 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 epoxy beads.

#### 1. Materials

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

- Epoxy beads (TAS8848N1110): 10 mg (Functional groups : Approx. 200 nmol/mg)
- Ligands: Approx. 30 mg

##### 1.2 Reagents

- N,N-Dimethylformamide (DMF) 15 mL
- Potassium carbonate (K<sub>2</sub>CO<sub>3</sub>) M.W. 138.21 50 mg
- Methanol 7 mL

##### 1.3 Apparatus

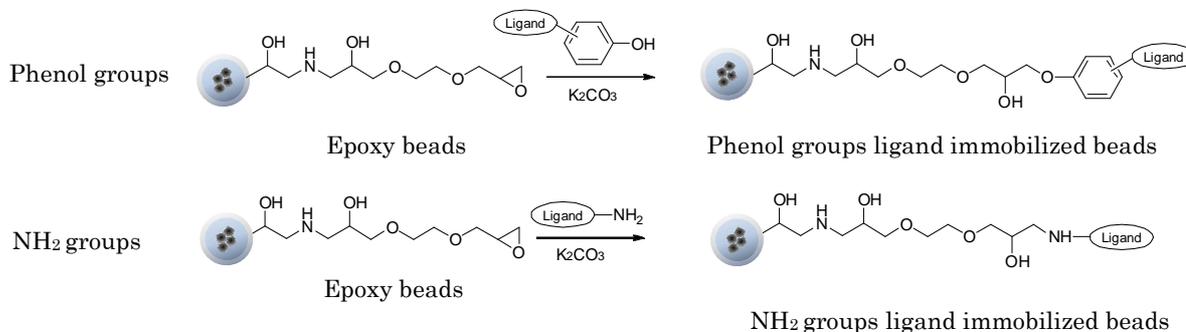
- Micro centrifuge
- Incubator
- Rotator
- Ultrasonic dispersing device

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

#### 2. Method

##### 2.1 Outline

The following is a schematic view of ligand immobilization. Both phenolic OH groups and NH<sub>2</sub> groups can be immobilized in the same method. Refer to the next section 2.2 “Procedures” for details.



##### 2.2 Procedures

- 1) Dissolve ligands (compounds) in DMF, and prepare 1 mL of 50 mM ligand solution.
- 2) Add 2.5 mg of epoxy beads (TAS8848N1110) 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 and the prepared ligand solution, and disperse the beads with an ultrasonic device. (Refer to the table on the next page.)
- 8) Add potassium carbonate of 10 mol times to ligands (e.g. 35 mg of potassium carbonate to 50 mM of ligands), and mix them with an ultrasonic device.

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(Or put the measured potassium carbonate into another tube, and add ligands and beads solution into the tube)

Concentration	(mM)	0	2	10	50
Epoxy beads	(mg)	2.5	2.5	2.5	2.5
DMF	( $\mu$ L)	500	480	400	0
50 mM ligand	( $\mu$ L)	0	20	100	500
Potassium carbonate	(mg)	0	1.4	7	35
Total	( $\mu$ L)	500	500	500	500

Note: If the ligand solution is added directly to the beads, the concentration could be locally raised. Add, therefore, the ligand solution after adding the DMF to the beads.

- 9) React them for 16 to 20 hours (overnight) at 60°C by using a rotator in an incubator. (The potassium carbonate is not dissolved.)
- 10) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.
- 11) Add 500 $\mu$ L of 50% DMF, and disperse the beads with an ultrasonic device.
- 12) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant. (As beads collect in a middle layer of the solution, discard the solution only in an upper layer.)
- 13) Repeat the above 11) to 12) one more time. (Wash the beads twice in total.)
- 14) Add 500 $\mu$ L of ultrapure water, 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) Add 500 $\mu$ L of 50% MeOH, and disperse the beads with an ultrasonic device.
- 17) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.
- 18) Repeat the above 16) to 17) two more times. (Wash the beads three times in total.)
- 19) Resuspend 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

- 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/af-notes.html> for moving pictures.)



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- Recover beads dispersed in DMF or 50% MeOH by centrifugation because the magnetic separation takes a longer time.
- Use DMF which is hydrated with a molecular sieve, or a low-moisture solvent.
- 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.