Experiment Protocol 014S

Immobilization of ligands (compounds with NH_2 groups) on NHS 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, 0.1 mM, 0.3 mM, and 1 mM when immobilizing ligands on NHS beads.

1. Materials

1.1 Beads and Ligands (Compounds)

- NHS beads (TAS8848N1141):4 mg (Functional groups : Approx. 200 nmol/mg)
- Ligands: Approx.1 mg

1.2 Reagents

- N,N-Dimethylformamide (DMF) 5 mL
- Amino ethanol M.W. 61.08 200 µL
- Triethylamine M.W. 101.19 1 µL
- Methanol (MeOH) 1.6 mL

1.3 Apparatus

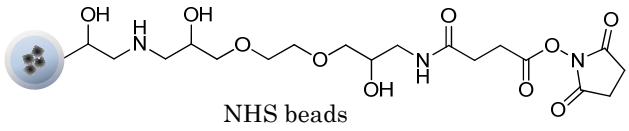
- Micro centrifuge
- Micro tube Mixer (TOMY MT-360, etc.)
- Ultrasonic dispersing device

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

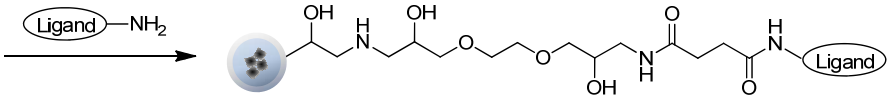
2. Method

2.1 Outline

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



NHS beads



NH_2 group ligand immobilized beads

2.2 Procedures

1) Dissolve ligands (compound) in DMF, and prepare 400 µL of ligand solution.
2) Dissolve amino ethanol in DMF, and prepare 1 mL of amino ethanol solution.
3) Add 1 mg of NHS beads into each of four micro-tubes. Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.
4) Add 80 µL of DMF, and disperse the beads. Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.
5) Add DMF and the prepared ligand solution, and disperse the beads with an ultrasonic device.

Concentration	(mM)	0	0.1	0.3	1
NHS beads	(mg)	1	1	1	1
DMF	(µL)	200	180	140	0
1mM ligands	(µL)	0	20	60	200
Total	(µL)	200	200	200	200

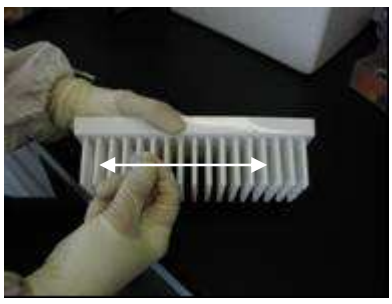
Note 1: If the ligand solution is added directly to the beads, the concentration could be locally raised. Add, therefore, the ligand solution after adding the DMF to the beads.

Note 2: When ligands are hydrochloride, add twice mole tri-ethyl amine to them. In this case, it will be easier to prepare 4 mM tri-ethyl amine and 2mM ligand solution, and mix them in equal volume.

6) React them for seventy minutes at room temperature by using a micro tube mixer.
7) Centrifuge at 15,000 rpm for five minutes at room temperature, and transfer the supernatant to a fresh micro-tube. (Supernatant A)
8) Add 200 µL of 1 M amino ethanol to the remaining beads each, and disperse the beads with anultrasonic dispersing device.
9) React them for two hours at room temperature by using a micro tube mixer. (Masking of ligand-non-binding carboxyl groups)
10) Centrifuge at 15,000 rpm for five minutes at room temperature, and transfer the supernatant to a fresh micro-tube. (Supernatant B)
11) Add 200 µL of 50% MeOH, 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) Resuspend in 40 µL of 50% MeOH, and store them at 4℃. (Concentration of ligand immobilized beads:0.5 mg/20 µL)

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/technique/affinity.html for moving pictures.)



Experiment Protocol 014S

- 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.
- You can investigate the following by determining quantity of succinimide in the supernatant A and B by means of HPLC.

  (Refer to Experiment Protocol 201 for the method.)

  A : The amount of immobilization of ligands (The amount of succinimide liberated when immobilizing ligands.)

  B : The amount of NHS groups on which ligands are not immobilized. (The amount of succinimide liberated when masking.)

  A+B : The amount of NHS groups of beads

- Use DMSO when ligands have low solubility in DMF. However, when quantify the amount of succinimide by HPLC using Experiment Protocol 201, decrease the concentration of DMSO down to 10% or less, for avoiding the peak of DMSO overlap the peaks of succinimide.