Experiment Protocol 109

Immobilization of ligands (alkyne structure compounds) on azide beads using click chemistry reaction

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. 0μM, 5μM, 25μM, and 125μM when immobilizing ligands on azide beads.

1. Materials

1.1 Beads and Ligands (Compounds)

- Azide beads (TAS8848N1160):10mg (Functional groups:Approx. 100nmol/mg)
- Ligands: Approx. 0.1mg

1.2 Reagents

- t-butyl alcohol (t-BuOH) 12mL
- Dimethylsulfoxide (DMSO) 3mL
- Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) M.W. 530.63 2.7mg
- Copper(Ⅱ)sulfate (CuSO4) M.W. 159.61 16mg
- (+)-Sodium L-ascorbate M.W. 198.11 20mg
- Methanol (MeOH) 4mL

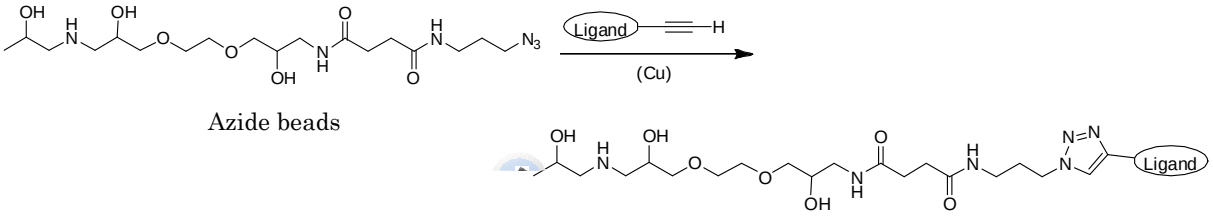
1.3 Apparatus

- 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.



Azide beads

Ligand immobilized beads

2.2 Preparation of solutions

1) Prepare 15mL of t-BuOH/DMSO solution by mixing 12mL of t- BuOH with 3mL of DMSO(*t*-BuOH : DMSO = 4:1)
2) Dissolve ligands (compounds) in *t*-BuOH/DMSO solution, and prepare 200μL of 500μM ligand solution.
3) Dissolve 2.7mg of TBTA in 1mL of *t*-BuOH/DMSO solution, and prepare 1mL of 5mM TBTA solution. Add 190μL of *t*-BuOH/DMSO solution to 10μL of 5mM TBTA, and prepare 200μL of 250μM TBTA solution.
4) Dissolve 16mg of $CuSO_4$ in 1mL of ultrapure water, and prepare 1mL of 100mM $CuSO_4$ solution. Add 190μL of ultrapure water to the 10μL of 100mM $CuSO_4$ solution, and prepare 200μL of 5mM $CuSO_4$ solution.

5) Dissolve 20mg of (+)-Sodium L-ascorbate in 1mL of ultrapure water, and prepare 1mL of 100mM (+)-Sodium L-ascorbate solution. Add 190µL of ultrapure water to 10µL of the 100mM (+)-Sodium L-ascorbate solution, and prepare 200µL of 5mM (+)-Sodium L-ascorbate solution.

6) Prepare 8mL of *t*-BuOH/DMSO/ultrapure water solution by mixing 4mL of the *t*-BuOH/DMSO solution prepared in the above 1) with 4mL of ultrapure water. (*t*-BuOH/DMSO solution: ultrapure water = 1:1)

2.3 Ligand immobilization

1) Add 2.5 mg of azide beads into each of four micro-tubes. Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.

2) Add 500µl of t-BuOH/DMSO solution, and disperse the beads. Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.

3) Repeat the above 2) two more times, and perform solvent displacement.

4) Add each reaction solution in the order as shown below, from the top solution Azide beads to the bottom Sodium ascorbate. In this case, after adding 250µM of TBTA, disperse the beads with an ultrasonic device. Then, add the remaining solutions.

Concentration	(µM)	0	5	25	125
Azide beads	(mg)	2.5	2.5	2.5	2.5
t-BuOH/DMSO	(µl)	250	240	200	0
500µM ligands	(µl)	0	5	25	125
250µM TBTA	(µl)	0	5	25	125
Ultrapure water	(µl)	250	240	200	0
5mM CuSO4	(µl)	0	5	25	125
5mM (+)-Sodium ascorbate	(µl)	0	5	25	125
Total	(µl)	500	500	500	500

5) React for 16 to 20 hours at room temperature by using a micro tube mixer.

6) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.

7) Add 500µl of t-BuOH/DMSO/ultrapure water solution, and disperse the beads with an ultrasonic device.

8) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.

9) Repeat the above 7) to 8) two more times. (Wash the beads with t-BuOH/DMSO/ultrapure water solution three times in total.)

10) Add 500µL of 50% MeOH, and disperse the beads with an ultrasonic device.

11) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.

12) Repeat the above 10) to 11) two more times. (Wash the beads three times in total.)

13) Disperse the beads in 100 µL of 50% MeOH, and store 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/af-notes.html for moving pictures.)

・ Recover beads dispersed in *t*-BuOH, DMSO, or 50% MeOH not by magnetic separation but by centrifugation because the magnetic separation takes a longer time.

・ 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.