Experiment Protocol　109S

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）:4 mg （Functional groups：Approx. 100 nmol/mg）
- Ligands: Approx. 0.1 mg

1.2 Reagents

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

1.3 Apparatus

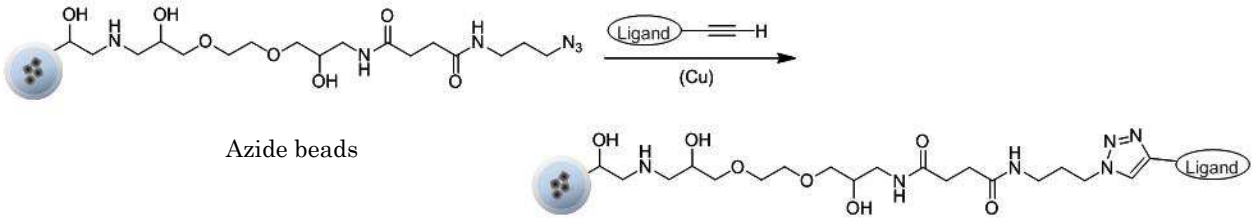
- Micro centrifuge
- Micro tube mixer（TOMY MT-360, etc.）
- Ultrasonic dispersing device

  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 Preparation of solutions

1) Prepare 15 mL of t-BuOH/DMSO solution by mixing 12 mL of t- BuOH with 3 mL of DMSO(*t*-BuOH : DMSO = 4:1)

   ※Since *t*-BuOH has a low freezing point (25.7 ℃), if the t-BuOH is solidified, please be dissolved before use.
2) Dissolve ligands (compounds) in *t*-BuOH/DMSO solution, and prepare 100 μL of 500 μM ligand solution.
3) Dissolve 2.7 mg of TBTA in 1 mL of *t*-BuOH/DMSO solution, and prepare 1mL of 5 mM TBTA solution. Add 95 μL of *t*-BuOH/DMSO solution to 5 μL of 5 mM TBTA, and prepare 100 μL of 250 μM TBTA solution.
4) Dissolve 16 mg of $CuSO_4$ in 1 mL of ultrapure water, and prepare 1 mL of 100 mM $CuSO_4$ solution. Add 95 μL of ultrapure water to the 5 μL of 100 mM $CuSO_4$ solution, and prepare 100 μL of 5 mM $CuSO_4$ solution.

5) Dissolve 20 mg of (+)-Sodium L-ascorbate in 1 mL of ultrapure water, and prepare 1 mL of 100 mM (+)-Sodium L-ascorbate solution. Add 95 µL of ultrapure water to 5 µL of the 100 mM (+)-Sodium L-ascorbate solution, and prepare 100 µL of 5 mM (+)-Sodium L-ascorbate solution.

6) Prepare 4 mL of *t*-BuOH/DMSO/ultrapure water solution by mixing 2 mL of the *t*-BuOH/DMSO solution prepared in the above 1) with 2 mL of ultrapure water. (*t*-BuOH/DMSO solution: ultrapure water = 1:1)

2.3 Ligand immobilization

1) Add 1 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 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 250 µM of TBTA, disperse the beads with an ultrasonic device. Then, add the remaining solutions.

Concentration	(µM)	0	5	25	125
Azide beads	(mg)	1	1	1	1
t-BuOH/DMSO	(µl)	100	96	80	0
500µM ligands	(µl)	0	2	10	50
250µM TBTA	(µl)	0	2	10	50
Ultrapure water	(µl)	100	96	80	0
5mM CuSO4	(µl)	0	2	10	50
5mM (+)-Sodium ascorbate	(µl)	0	2	10	50
Total	(µl)	200	200	200	200

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

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

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

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

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

8) Add 200 µL of 50% MeOH, and disperse the beads with an ultrasonic device.

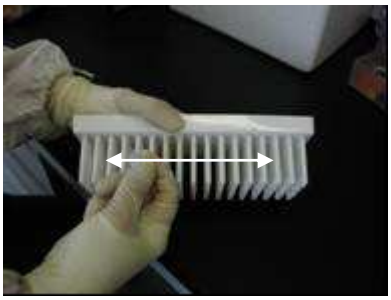
9) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.

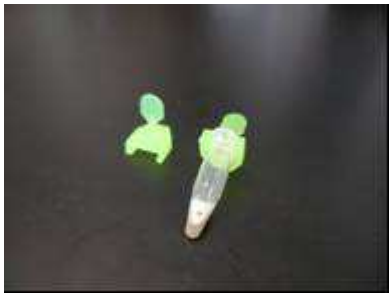
10) Repeat the above 10) to 11) two more times. (Wash the beads three times in total.)

11) Disperse the beads in 40 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/technique/affinity.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.