

Experiment Protocol 015

Immobilization of MTX aminated derivatives on NHS beads

1. Materials

1.1 Beads, MTX aminated derivatives

- NHS beads(TAS8848N1141) 4 mg (1mg/condition)
Functional groups : Approx. 200 nmol/mg
- MTX derivative (TAS8849N101)

1.2 Reagents

- N,N-Dimethylformamide (DMF) 3 mL
- Triethylamine M.W. 101.19 7 μ L
- Amino ethanol M.W. 61.08 61 μ L
- Methanol (MeOH) 3 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

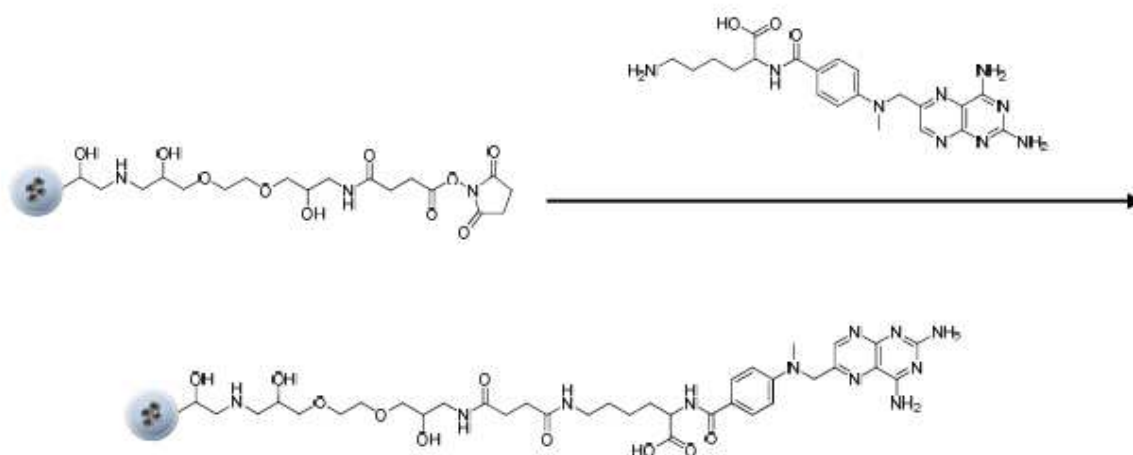
(TAITEC), and an ultrasonic dispersing device: TA4905 (Tamagawa Seiki).

2. Method

2.1 Preparation for reagent solutions

- 1) Add 1mL of DMF to one bottle of MTX aminated derivative to prepare 0.2mM of MTX aminated derivative solution. Fully dissolve the DMF added MTX aminated derivative by using an ultrasonic homogenizer or an ultrasonic dispersing device.
- 2) Dissolve 7 μ l of triethylamine in 993 μ l of DMF to prepare 1 ml of 50 mM triethylamine solution. Add 5 μ l of DMF to 5 μ l of 50 mM triethylamine solution to prepare 10 μ l of 25 mM triethylamine solution. In addition, add 5 μ l of DMF to 5 μ l of 25 mM triethylamine solution to prepare 10 μ l of 12.5 mM triethylamine solution.
- 3) Dissolve 61 μ l of amino ethanol in 939 μ l of DMF to prepare 1 ml of 1M amino ethanol solution.

2.2 Immobilization of MTX



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- 1) 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.
- 2) Add 100 μ L of DMF, and disperse the beads. Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.
- 3) Add DMF and the prepared ligand solution as shown below, and disperse the beads with an ultrasonic device.

Concentration (mM)	0	0.05	0.1	0.2
NHS beads (mg)	1	1	1	1
0.2mM MTX aminated derivative (μ l)	0	50	100	200
Triethylamine (μ l)	0	2 (12.5mM)	2 (25mM)	2 (50mM)
DMF (μ l)	200	148	98	0
Total (μ l)	200	200	200	202

※ Add 12.5mM, 25mM, and 50mM triethylamine prepared in the above 2.1 to each concentration respectively.

- 4) React them for seventy minutes at room temperature by using a micro tube mixer.
- 5) After the reaction, centrifuge at 15,000rpm for five minutes at room temperature, and transfer the supernatant to a fresh micro-tube. (Supernatant A)
- 6) Add 200 μ L of 1 M amino ethanol to the remaining beads each, and disperse the beads with an ultrasonic dispersing device.
- 7) React them for two hours at room temperature by using a micro tube mixer.
(Masking of ligand-non-binding carboxyl groups)
- 8) After the masking, centrifuge at 15,000rpm for five minutes at room temperature, and transfer the supernatant to a fresh micro-tube. (Supernatant B)
- 9) Add 200 μ L of 50% MeOH, and disperse the beads with an ultrasonic dispersing device.
- 10) Centrifuge at 15,000 rpm for five minutes at room temperature, and discard the supernatant.
- 11) Repeat the above 9) to 10). (Wash the beads three times in total.)
- 12) Resuspend in 40 μ L of 50% MeOH, and store them at 4°C. (Concentration of ligand immobilized beads:0.5mg/20 μ L)

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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 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.