Protocol 014

Immobilization of Chemical Compounds on NHS Beads

Materials

1) Beads and Ligands

NHS beads (Product No.: TAS8848N1141): 10 mg
(Functional groups: 200–300 nmol/mg)
NHS beads are stored in IPA (isopropyl alcohol)
Chemical compounds: approximately 1 mg

*** Please keep in mind that the shelf life of NHS beads are <1 mo. Prolonged storage will result in loss of NHS esters impacting loading capacity.**

2) Reagents

N,N-Dimethylformamide (DMF)
2-Aminoethanol (ethanolamine) (M.W. 61.08)
Methanol
Ammonium acetate (M.W. 77.08)
Acetic acid (M.W. 60.05)
Acetonitrile

3) Apparatus

Micro centrifuge (HITACHI CF15RX2)
Microtube Mixer (TOMY MT-360)
Ultrasonic homogenizer (TAITEC VP-15 with cup horn)
HPLC system (Waters 2695 with 2998 PDA and SMH)
HPLC column (Waters Symmetry 5μm C18 4.6×250mm WAT054275)

Methods

1) Immobilization of Chemical Compound on NHS Beads

Dissolve chemical compound in DMF

Prepare 5 mM chemical compound solution in DMF

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Start with 2.5 mg of NHS beads for each immobilization concentration

(NHS beads are suspended in IPA)

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Wash beads with DMF

(Centrifuge at 15,000 rpm for 5 min at R.T. and remove the supernatant)

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Suspend beads in each amount of DMF, and add each amount of 5 mM chemical compound solution as shown below

Conc. of chemical compound at immobilization (mM)	0	0.1	0.3	1
DMF (μl)	500	490	470	400
5 mM chemical compound solution (μl)	0	10	30	100

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Mix for 70 min at R.T. by using Microtube Mixer

↓

Centrifuge at 15,000 rpm for 5 min at R.T.

↓

Transfer the supernatant (Sup A) to another tube for measurement of released NHS by HPLC [refer to Method 2) below for measurement instructions]

↓

Resuspend in 500 μl of 1 M ethanolamine in DMF (by using homogenizer)

↓

Mix for 2 h at R.T. by using Microtube Mixer

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Centrifuge at 15,000 rpm for 5min at R.T.

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Transfer the supernatant (Sup B) to another tube for measurement of released NHS by HPLC [refer to Method 2) below for measurement instructions]

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Wash with 500 μl of 50 % methanol in H_2O, 3 times

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Resuspend in 100 μl of 50 % methanol in H_2O (by using homogenizer)

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Store at 4 °C

2) Determination of the Amount of Immobilized Chemical Compound

Prepare 10 mM ammonium acetate (pH 5.70)

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Prepare sample buffer and standard samples

Sample buffer: 47 ml of 10 mM ammonium acetate + 3 ml of acetonitrile (94 : 6)

Standard sample: 0, 10, 100, 1000, 5000 μM NHS in DMF

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Mix 20 μl of sample (standard sample, Sup A, or Sup B) and 180 μl of sample buffer

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Filter at 5,000 ×g for 1 min by using 0.2 μm centrifugal filter devices

↓

Place 150 μl of the filtrate in autosampler of HPLC system

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Set HPLC conditions

- Gradient (40 min including column washing process):

Time (min)	10 mM ammonium acetate (%)	Acetonitrile (%)	Gradient curve
0	94	6	-
10	60	40	Linear
12	20	80	Linear
20	20	80	Linear
22	94	6	Linear
40	94	6	Linear

- Flow Rate: 1 ml/min
- Injection Volume: 50 μl
- Temperature: 40 °C

↓

Run HPLC

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Analyze the amount of NHS in the samples from peak area at 260 nm

↓

Measure the amount of released NHS in Sup A and Sup B

↓

Amount of immobilized chemical compound is calculated from measurement of Sup A

(Amounts of carboxyl groups on COOH beads are calculated from measurement of Sup A and Sup B)