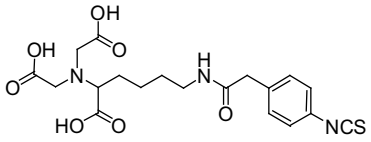
Isothiocyanobenzyl-NTA Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/i279.pdf

N-[5-(4-Isothiocyanatobenzyl)amido-1-carboxypentyl]iminodiacetic acid
Molecular Weight: 437.47
Formula: $C_{19}H_{23}N_3O_7S$

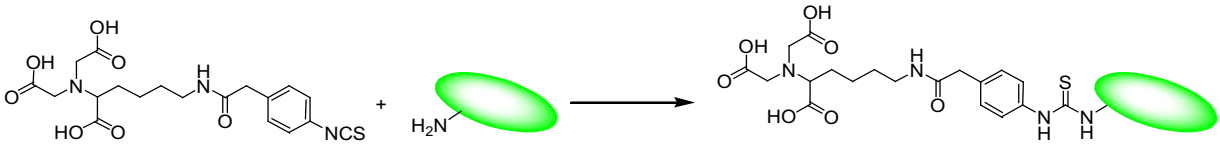


General Infomation

Isothiocyanobenzyl-NTA* (ITC-Bz-NTA) is used for the introduction of a chelating function to various materials such as amine compound, biomolecules, and surfaces through their amino groups under physiological conditions. Since NTA makes a stable chelate with heavy metal ions, NTA-conjugated compound, biomolecules, and surfaces can hold heavy metals. Such heavy metal ion chelate can be used for detection or separation of specific substances that interact with heavy metal ions.

∗ NTA : Nitrilotriacetic acid

ITC-Bz-NTA Labeling Reaction



ITC-Bz-NTA + **Protein or DNA** → **NTA-conjugate**

Storage Condition

Store at -20℃ .

General Labeling Protocol: Protein

ImmunoglobulinG (IgG)

1. Prepare a 1 mg/ml IgG solution with 100 mmol/l Bicine Buffer (pH 8.5).
2. Dissolve 1 mg ITC-Bz-NTA with 50 µl DMSO.
   ∗ The DMSO solution is stable for 1 week at -20℃ .
3. 100 µl IgG solution and 10 µl ITC-Bz-NTA solution in a microtube.
4. Incubate the mixture at 37℃ for 1 hour.
5. Purify the NTA-conjugate with a gel filtration or a dialysis.

Labeling profile of IgG with ITC-Bz-NTA

Concentration (NTA)	Labeling ratio (NTA/IgG)
250 µmol/l	1
500 µmol/l	3
1000 µmol/l	5

The labeling ratio of ITC-Bz-NTA to IgG was determined by TNBS(2,4,6-Trinitrobenzenesulfonic acid) method.

IgG : 6.6 µmol/l

General Labeling Protocol: DNA

DNA oligomer with an amino group

1. Prepare a DNA oligomer solution with 100 mmol/l Bicine buffer(pH 8.5).
2. Dissolve 1 mg ITC-Bz-NTA with 50 µmol/l DMSO*.
   ∗ The DMSO solution is stable for 1 week at -20℃ .
3. Mix the DNA oligomer solution and ITC-Bz-NTA solution in a microtube[a)].
4. Incubate the mixture at 37℃ for 1 hour.
5. Purity the NTA-conjugated with a gel filtration.
   a) Generally, 20 times equivalent of ITC-Bz-NTA should be added to the DNA oligomer.
   The volume of the DMSO solution should be less than 20% of the DNA oligomer solution.

Preparation of Ni Complex

Preparation of Ni-NTA complex

1. Prepare $NiCl_2$ solution with 10 mmol/l HCl.
2. Add the 5 equivalent of Ni solution.
3. Adjust the pH at 7 with the 0.1 mol/l $NaHCO_3$.

Caution

An appropriate number of NTA to be introduced will differ optimize the protocol to prepare a suitable NTA-conjugated materials.

If you need more infomation, please contact Dojindo technical service.

Dojindo Laboratories
2025-5 Tabaru, Mashiki-machi, Kamimashiki-gun, Kumamoto
861-2202, Japan Phone: +81-96-286-1515 Fax: +81-96-286-1525
E-mail: info@dojindo.co.jp Web: www.dojindo.co.jp

Dojindo Molecular Technologies,Inc.
Tel: +1-301-987-2667 Web:http://www.dojindo.com/
Dojindo EU GmbH
Tel: +49-89-3540-4805 Web: http://www.dojindo.eu.com/
Dojindo China Co., Ltd
Tel: +86-21-6427-2302 Web:http://www.dojindo.cn/