

Biotin-X-X-NHS

Cat. Nos. BXX51005 and BXX51010

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1. Introduction

Biotin-X-X-NHS 6-[(+)-Biotinamidocaproylamido] caproic acid N-hydroxysuccinimide ester (also referred to as Biotin-LC-LC-NHS) is a water-soluble, amine-reactive ester of biotin that can be used to label proteins or nucleic acids containing aliphatic amino groups. For example, in aqueous solution, the N-hydroxysuccinimide (NHS) group of the biotin-X-X-NHS rapidly and with high efficiency reacts with the amine group of the aminoallyl-nucleotides incorporated into RNA during an *in vitro* transcription reaction or into DNA during a reverse transcription or DNA-dependent DNA polymerase reaction.

Epicentre's Biotin-X-X-NHS is supplied as a dry powder in an air-tight vial with a self-sealing septum. The compound has been packaged under dry inert gas and is stable for at least one year when stored in powdered form in the air-tight vial at 4°C.

Applications

- Biotinylation of aminoallyl-aRNA (cRNA) for preparing labeled target for hybridization to microarrays.
- Biotinylation of aminoallyl-cDNA for preparing labeled target for hybridization to microarrays.
- Biotinylation of aminoallyl-RNA or aminoallyl-DNA for use as non-radioactive probe for *in situ* hybridization or blotting experiments.
- Biotinylation of proteins.

2. Product Specifications

Storage: Store desiccated at 4°C

Molecular Formula: C₂₆H₁₄N₅SO₇

Molecular Weight: 567.7

Spacer Arm: 30.5 Angstroms

Formulation: White powder

Melting point: 149°C-150°C

Quality Control: Rf = 0.43 as determined by TLC on silica gel 60 using CH₂Cl₂:Methanol (88:12).

3. Kit Contents

Cat. #	Quantity
Biotin-X-X-NHS	
Supplied in septum-stoppered vials of 2.5 mg per vial.	
BXX51005	5 Vials (5 x 2.5 mg)
BXX51010	10 Vials (10 x 2.5 mg)

4. Related Products

The following products are also available:

- TargetAmp™ Aminoallyl-aRNA Amplification Kits
- Aminoallyl-UTP Solution

5. Biotinylation of Aminoallyl-RNA

General Considerations

Biotin-X-X-NHS is readily hydrolyzed by water and can react with nucleophilic compounds, such as those with amino groups such as Tris buffers. Biotin-X-X-NHS should be dissolved in dry dimethyl sulfoxide (DMSO) as close to the time of use as possible. Once dissolved in DMSO or other solvent, its stability is entirely dependent on the continued absence of water or other nucleophilic compounds. Since DMSO is extremely hygroscopic and quickly takes up water vapor from the air, we recommend using Biotin-X-X-NHS that has been freshly dissolved in dry DMSO. If the Biotin-X-X-NHS has been dissolved and stored in DMSO, the stability of the Biotin-X-X-NHS should be validated prior to using it for biotinylation of aminoallyl-RNA from a rare or precious sample.

Preparation and Additionally Required Reagents and Materials

1. **NaHCO₃ Buffer:** Prepare a 25 mg/ml solution of sodium bicarbonate (NaHCO₃), pH 8.5. Store in aliquots at –20°C for up to 6 months. Do not reuse thawed solutions. Check the pH of the buffer before each use. If the pH is not at 8.5-8.8, prepare a fresh solution.
2. **Anhydrous Dimethyl Sulfoxide:** EPICENTRE has had the most experience with, and recommends anhydrous DMSO from Acros Organics (distributed by Fisher Scientific; cat. no. 34844100).
3. **4 M Hydroxylamine:** Dissolve 5.56 g Hydroxylamine-HCl (e.g., Sigma cat. no. 159417) in 8 ml of RNase-Free water. Titrate to pH 8.5-8.8 using 10 M NaOH. Adjust the volume to 20 ml with RNase-Free water. Store at –20°C for up to 6 months.
4. Microcon® YM-30 Centrifuge Filter Device: (Millipore cat. No. 42422; 1 device per sample) - **or** - RNeasy® Mini Kit: (Qiagen cat. no. 74104; 1 column per sample) - **or** - RNeasy® MinElute® Cleanup Kit: (Qiagen cat. No. 74204; 1 column per sample). See “Purification of Biotinylated-RNA” for details.
5. Aminoallyl-RNA Sample: The aminoallyl-RNA sample should be purified free of unincorporated aminoallyl-NTP and buffers containing amine salts such as Tris-HCl (e.g., by using the RNeasy® Mini Kit from Qiagen). The aminoallyl-RNA should be dissolved to at least 2 mg/ml in RNase-Free water.

6. Procedures

A. Biotinylation Procedure

The biotin conjugation reaction described here has been optimized for labeling 20-35 μg of aminoallyl-RNA. Scale the labeling reaction up or down depending on the amount of aminoallyl-RNA to be labeled.

1. Make sure the anhydrous DMSO is at room temperature before opening the bottle. Using a dry syringe, add 250 μl of the room temperature, anhydrous DMSO to a septum-sealed vial containing 2.5 mg of Biotin-X-X-NHS and mix to dissolve. Each 2.5-mg vial of Biotin-X-X-NHS can be used to label up to 50 samples of aminoallyl-RNA comprising up to 35 μg of aminoallyl-RNA in each sample.

Important! DMSO is hygroscopic. Make sure the DMSO is at room temperature before opening and then seal the bottle immediately after use. Biotin-X-X-NHS should be used immediately after dissolving in DMSO.

2. For every 20-35 μg of aminoallyl-RNA, combine the following at room temperature:

x μl	RNase-Free Water
x μl	(20-35 μg) Aminoallyl-RNA at ≥ 2 mg/ml
5 μl	NaHCO_3 Buffer
5 μl	Biotin-X-X-NHS in DMSO
10 μl	DMSO
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30 μl	Total reaction volume

Note: The stability of Biotin-X-X-NHS in DMSO has not been tested by Epicentre. Therefore, we recommend discarding any remaining material.

3. Mix the reaction well. Incubate for 1 hour at room temperature protected from the light, with occasional mixing.
4. Terminate and quench the reaction by adding 13.5 μl of 4 M Hydroxylamine (pH 8.5-8.8). Incubate at room temperature for 15 minutes.

B. Purification of Biotinylated-RNA

Biotinylated-RNA can be purified using a Microcon YM-30 Centrifuge Filter Device or the RNeasy Mini or MinElute Kits. In general, recovery of the biotinylated-RNA is greatest with the Microcon YM-30 device. A procedure for using this device is provided below.

If using the RNeasy kits:

- 1) Use the Qiagen MinElute Cleanup Kit when purifying < 40 μg , of biotinylated-RNA. Use the Qiagen RNeasy Mini Kit when purifying > 40 μg , of biotinylated-RNA.
- 2) When using either kit, follow the manufacturer's procedure, except, recover the biotinylated-RNA by performing the elution step twice in order to maximize recovery.

Using the Microcon YM-30 Centrifuge Filter Device:

1. Pre-rinse the Microcon YM-30 filters with 200 μ l of RNase-Free water.
2. Add 400 μ l of RNase-Free water to the sample.
3. Apply the sample to the filter and then place the filter into a 1.5 ml collection tube.
4. Process the sample according to the Microcon YM-30 manufacturer's instructions. Discard the flow-through.
5. Add an additional 400 μ l of RNase-Free water to the filter. Process the sample according to the Microcon YM-30 manufacturer's instructions. Discard the flow-through.
6. Repeat step 5 two times.
7. Collect the purified biotin-RNA sample per manufacturer's instructions.

C. Quantifying the Concentration and Yield of the Biotinylated-RNA

1. Prepare a 1:100-1:200 dilution of the biotinylated-RNA into the minimum volume of water or TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) required by the spectrophotometer cuvette that will be used.
2. Zero the spectrophotometer at 260 nm using the diluent (water or TE buffer) that was used to dilute the biotinylated-RNA sample.
3. Measure and record the absorbance of the diluted biotinylated-RNA at 260 nm (A_{260}).
4. Calculate the concentration of the biotinylated-RNA.
An A_{260} reading of 1.0 is equal to an RNA concentration of 40 μ g/ml.
Biotinylated-RNA concentration = (A_{260} reading) x (dilution factor) x (40 μ g/ml)
Example: Dilution for A_{260} measurement = 1:200; A_{260} of the 1:200 dilution = 0.175
Biotinylated-RNA concentration = (0.175) x (200) x (40 μ g/ml) = 1,400 μ g/ml (1.4 μ g/ μ l)
5. Calculate the yield of biotinylated-RNA using the formula:
Yield = (biotinylated-RNA concentration) x (volume of biotinylated-RNA recovered)
Example: Concentration of biotinylated-RNA = 1.4 μ g/ μ l; Volume recovered = 20 μ l
Yield of biotinylated-RNA = (1.4 μ g/ μ l) x (20 μ l) = 28 μ g of biotinylated-RNA
Note: In this example, if 1 μ l of 1.4 μ g/ μ l biotinylated-RNA was used for the spectrophotometer reading, then there are now 26.6 μ g of biotinylated-RNA remaining in the tube.

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