

Pierce™ RNA 3' End Desthiobiotinylation Kit

20163

2508.0

Number	Description
20163	Pierce RNA 3' End Desthiobiotinylation Kit, sufficient for 20 biotinylation reactions

Kit Contents:

T4 RNA Ligase Reaction Buffer (10X), 100µL, 0.5M Tris•HCl, 0.1M MgCl₂, 0.1M DTT, 10mM ATP; pH 7.8 at 25°C

T4 RNA Ligase (20,000U/mL), 40µL

Non-labeled RNA Control (10µM), 100µL

5'-CCUGGUUUUAAGGAGUGUCGCCAGAGUGCCGCGAAUGAAAAA-3'

Biotinylated IRE RNA Control (125nM), 35µL

5'-UCCUGCUUCAACAGUGCUUGGACGGAAC-3'-Biotin

RNase Inhibitor (40U/µL), 2 × 10µL

Nuclease-free Water, 1.5mL

DMSO, 200µL

PEG 30%, 300µL

Desthiobiotinylated Cytidine (Bis)phosphate, (1mM), 40µL

Glycogen (20mg/mL), 20µL

Storage: Upon receipt store at -20°C. Product shipped on dry ice.

Introduction

The Thermo Scientific Pierce RNA 3' End Desthiobiotinylation Kit uses T4 RNA ligase to attach a single biotinylated nucleotide to the 3' terminus of an RNA strand. The unique feature of this kit is the desthiobiotinylated cytidine bisphosphate nucleotide that contains a 3',5' phosphate on the ribose ring to accommodate the ligation and a desthiobiotin linker on the cytidine for detection. The kit contains T4 RNA ligase reaction components; a positive control, non-labeled RNA strand and a biotinylated RNA probe to quantitate labeling. To enhance biotinylation efficiency and RNA stability, RNase inhibitor, glycogen and ligation-enhancing reagents are also included. Once biotinylated, the labeled RNA probe is easily prepared for use in downstream applications such as RNA electrophoretic mobility shift assays (Thermo Scientific LightShift Chemiluminescent RNA EMSA Kit, Product No. 20158), RNA pull-down assays (Thermo Scientific Pierce Magnetic RNA Pull-Down Kit, Product No. 20164) or miRNA profiling.

Regulation of cellular function is dependent on critical RNA interactions with proteins and other RNA, including miRNA. These interactions have been difficult to isolate and are highly dependent on maintaining RNA secondary structure. To enrich for these interactions, it is often necessary to label the RNA; however, non-radioactive labeling methods can compromise RNA secondary structure or interfere with protein-RNA interactions. The Pierce RNA 3' End Desthiobiotinylation Kit provides a rapid non-radioactive method for RNA labeling that results in minimal interference of RNA secondary structure. The optimized labeling conditions accommodate RNA of different sizes, complexity and sources and produce ligation efficiencies of > 70%.

Additional Materials Required

- 1-50pmol of RNA for labeling
- Heated mixer/chiller for incubation at 37°C/16°C
- Chloroform:isoamyl alcohol (24:1)
- Nuclease-free pipette tips and tubes
- Microcentrifuge
- Heating block
- 5M NaCl
- Ultrapure water
- 100% ethanol, ice-cold
- 70% ethanol, ice-cold

Procedure for RNA Ligation

Note: This kit is optimized for 50pmol of RNA; however, RNA amounts from 1-50pmol generally produce similar ligation efficiencies. Purified RNA produces the most robust results. Optimize as needed to achieve the best efficiency.

Note: Maintain a nuclease-free environment during this procedure and when working with the RNA intended for labeling.

A. RNA Ligation Reaction

1. Thaw all kit components except the PEG 30% and DMSO on ice. Thaw DMSO at room temperature and warm the PEG 30% at 37°C for 5-10 minutes until volume is fluid.
2. Adjust the heating block to 85°C.
3. Transfer 5µL of the Non-labeled RNA Control to a microcentrifuge tube. Heat the RNA for 3-5 minutes at 85°C. Place RNA immediately on ice.

Note: The RNA may require heating to relax the secondary structure. Also, heating the RNA in the presence of ~25% DMSO may increase efficiency for RNA with significant secondary structure.

4. Prepare the labeling reaction for the control system or test RNA by adding components in the order listed in Table 1.

Note: The last added reagent is PEG 30%. Carefully pipette the PEG 30% into the reaction mixture. Use a new pipette tip to mix the ligation reaction after the PEG 30% addition.

Table 1. Components and preparation of the RNA ligation reaction.

<u>Component</u>	<u>Volume (µL)</u>	<u>Final Concentration</u>
Nuclease-free Water	3	---
10X RNA Ligase Reaction Buffer	3	1X
RNase Inhibitor	1	40U
Non-labeled RNA Control or Test RNA	5	50pmol
Biotinylated Cytidine Bisphosphate	1	1nmol
T4 RNA Ligase	2	40U
PEG 30%	15	15%
Total	30	---

5. Incubate the reactions at 16°C for 2 hours for the control RNA. Ligation may require overnight incubation to increase efficiency.
6. Add 70µL of nuclease-free water to the ligation reaction.

7. Add 100 μ L of chloroform:isoamyl alcohol to each reaction to extract the RNA ligase. Vortex the mixture briefly, then centrifuge 2-3 minutes at high speed in a microcentrifuge to separate the phases. Carefully remove the top (aqueous) phase and transfer to a nuclease-free tube.
8. Add 10 μ L of 5M NaCl, 1 μ L of glycogen and 300 μ L of ice-cold 100% ethanol. Precipitate for \geq 1 hour at -20°C.
9. Centrifuge at \geq 13,000 \times g for 15 minutes at 4°C. Carefully remove the supernatant, taking care not to disturb the pellet.
10. Wash the pellet with 300 μ L of ice-cold 70% ethanol. Carefully remove ethanol and air-dry the pellet (~5 minutes).
11. Resuspend the pellet in 20 μ L of nuclease-free water or buffer of choice.

Procedure for Determining Labeling Efficiency by Dot Blotting

To detect the biotinylated RNA, the following protocol uses the Thermo Scientific Chemiluminescent Nucleic Acid Detection Module Kit (Product No. 89880), which is available separately.

1. Dilute the Biotinylated IRE RNA Control 25-fold in ultrapure water to make a 5nM stock solution (e.g., 2 μ L of RNA and 48 μ L of ultrapure water).
2. In microcentrifuge tubes, prepare a series of RNA standards according to the table below.

Table 2. Preparation of the RNA standards.

<u>Component (μL)</u>	<u>Biotin (%)</u>				
	100	75	50	25	0
Biotinylated IRE RNA Control	12	9	6	3	0
Water	48	51	54	57	60
Total Volume (μL)	60	60	60	60	60

3. Add 50 μ L of the Biotinylated IRE RNA Control stock solution into wells A1-A5 of a 96-well plate.
4. In a microcentrifuge tube, make a 250-fold dilution of the test RNA labeling reaction in ultrapure water to achieve a final concentration of 10nM (e.g., 1 μ L of test RNA and 249 μ L of water).
5. Place 50 μ L of each 10nM test RNA sample into unused “A” wells of the 96-well plate. Prepare a series of two-fold dilutions by removing 25 μ L from all “A” wells and mixing them with 25 μ L of ultrapure water in corresponding “B” wells; continue down the plate through the “E” wells.
6. Spot 2 μ L of the Biotinylated IRE RNA Control and test RNA onto a positively charged nylon membrane and allow it to absorb.
7. Immediately UV crosslink the membrane by one of the following three methods:
 - Option 1:** Use a commercial UV-light crosslinking instrument equipped with 254nm bulbs (45-60 second exposure using the auto-crosslink function) and crosslink at 120mJ/cm².
 - Option 2:** Use a hand-held UV lamp with 254nm bulbs positioned ~0.5cm from the membrane and crosslink for 5 minutes.
 - Option 3:** Place the membrane face down on a transilluminator equipped with 312nm bulbs and crosslink for 10-15 minutes.
8. Immediately continue with the detection analysis or store the membrane dry at room temperature.
9. Detect the spotted RNA controls and test samples using the Chemiluminescent Detection Module. To determine labeling efficiency, compare spot intensities of the test RNA to the Biotinylated IRE RNA Controls.
10. For quantitation, identify the non-saturated spots with the same RNA concentration for the Biotinylated IRE RNA Control and test RNA for spot densitometry.

Note: Typical labeling efficiencies of the control RNA are \geq 75%. Labeling efficiency varies depending on the length and complexity of the RNA. Typically, RNA with low labeling efficiency (~20%) will produce adequate signal intensity in chemiluminescent EMSAs. Use RNA probes with \geq 75% labeling in the low nanomolar range. Probes with lower labeling efficiency will require a higher concentration.

Troubleshooting

Problem	Possible Cause	Solution
Inefficient ligation	RNA quality was poor	Gel purify RNA
	RNA had complex secondary structure	Heat at 85°C with 25% DMSO for 5 minutes and fast cool
		Increase incubation time and temperature
		Optimize DMSO concentration
	RNA was not within the proper concentration range	Determine RNA concentration by $A_{260/280}$ absorbance measurement and convert to pmol as follows: $(\mu\text{g} \times 10^{-6})(1 \times 10^{12} \text{ pmol/mol}) / (330\text{g/mol}) (\# \text{ bases}) = \text{pmol nucleic acid}$
	<i>In vitro</i> transcription reaction was inefficient or produced multiple transcripts	Before labeling, evaluate RNA by gel electrophoresis
	Improper ligation ratio was used	Determine RNA concentration by $A_{260/280}$ absorbance measurement and increase or decrease the amount of biotinylated nucleotide or RNA
	Ligation time was not sufficient	Some RNAs effectively ligate within 30 minutes; highly complex and lengthy RNAs will require longer incubation (e.g., overnight)
Ligation temperature was not optimal	Increase incubation temperature to 37°C	
Degraded RNA	A nuclease-free environment was compromised	Clean work area
		Ensure all plastics are from unopened packages
	mRNA from <i>in vitro</i> transcription reaction was degraded	After transcription, ensure RNA is intact by gel electrophoresis
Non-functional RNA in downstream application	RNA was improperly folded	Re-fold RNA by heating at 85-90°C for 2-5 minutes and slowly cool to room temperature

Related Thermo Scientific Products

20164	Pierce Magnetic RNA Pull-Down Kit
20158	LightShift™ Chemiluminescent RNA EMSA Kit
89880	Chemiluminescent Nucleic Acid Detection Module Kit
89880A	Nucleic Acid Detection Blocking Buffer
20159	tRNA
77016	Biodyne™ B Nylon Membranes, 0.45µm, 8cm × 12cm, 25 sheets

General References

- England, T.E., *et al.* (1980). Specific labeling of 3' termini of RNA with T4 RNA ligase. *Methods Enzym* **65**:65-74.
- Keith, G. (1983). Optimization of conditions for labeling the 3' OH-end of tRNA using T4 RNA ligase. *Biochimie* **65**:367-70.
- McKinley, B.A. and Sukhodolets, M.V. (2007). *Escherichia coli* RNA polymerase-associated SWI/SNF protein RapA: Evidence for RNA-directed binding and remodeling activity. *Nucleic Acids Res* **35**:7044-60.

Biodyne is a trademark of Pall Corporation.

Products are warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation"). No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than Buyer. Any model or sample furnished to Buyer is merely illustrative of the general type and quality of goods and does not represent that any Product will conform to such model or sample.

NO OTHER WARRANTIES, EXPRESS OR IMPLIED, ARE GRANTED, INCLUDING WITHOUT LIMITATION, IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR ANY PARTICULAR PURPOSE, OR NON INFRINGEMENT. BUYER'S EXCLUSIVE REMEDY FOR NON-CONFORMING PRODUCTS DURING THE WARRANTY PERIOD IS LIMITED TO REPAIR, REPLACEMENT OF OR REFUND FOR THE NON-CONFORMING PRODUCT(S) AT SELLER'S SOLE OPTION. THERE IS NO OBLIGATION TO REPAIR, REPLACE OR REFUND FOR PRODUCTS AS THE RESULT OF (I) ACCIDENT, DISASTER OR EVENT OF FORCE MAJEURE, (II) MISUSE, FAULT OR NEGLIGENCE OF OR BY BUYER, (III) USE OF THE PRODUCTS IN A MANNER FOR WHICH THEY WERE NOT DESIGNED, OR (IV) IMPROPER STORAGE AND HANDLING OF THE PRODUCTS.

Unless otherwise expressly stated on the Product or in the documentation accompanying the Product, the Product is intended for research only and is not to be used for any other purpose, including without limitation, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses, or any type of consumption by or application to humans or animals.

Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2012 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.