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Decade™ Markers System

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Contents	Quantity	Storage conditions
Decade™ Marker RNA (100 ng/µL in 10 mM Tris, pH 7)	10 µL	Store at –80°C.
10X Cleavage Reagent[1]	200 µL	Store at room temperature.
T4 Polynucleotide Kinase (10 U/µL)	10 µL	Store at 4°C or –20°C, as indicated on the label.
10X Kinase Reaction Buffer	10 µL	
Gel Loading Buffer II	1.4 mL	
Nuclease-free Water	1 mL	

[1] The 10X Cleavage Reagent is volatile. Tighten the cap after use to avoid evaporation.

Note: This product is shipped on dry ice.

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product description

The Decade™ Markers System produces a set of radiolabeled RNA molecular weight markers 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10 nucleotides in length. The Decade™ Marker System contains all of the reagents necessary to produce radiolabeled RNA molecular weight markers, except [γ–^{32}P]ATP.

The Decade™ Markers are derived from cleavage of a single 150-nt gel-purified transcript, Decade™ Marker RNA. The transcript is 5′ end-labeled in a kinase reaction with [γ–^{32}P]ATP, specific activity 3000 Ci/mmol or greater. The labeled transcript is then diluted into a cleavage reagent that generates the molecular weight marker set. Because the cleavage reaction does not go to completion, some original transcript remains, providing a marker at 150 nt in addition to the ladder generated; see Figure 1.

The labeling, cleavage, and gel preparation steps can be completed in about 1 hour. Decade™ Markers from a single reaction can be used for 5–20 experiments, depending on how much radioactivity is required for the experiment.

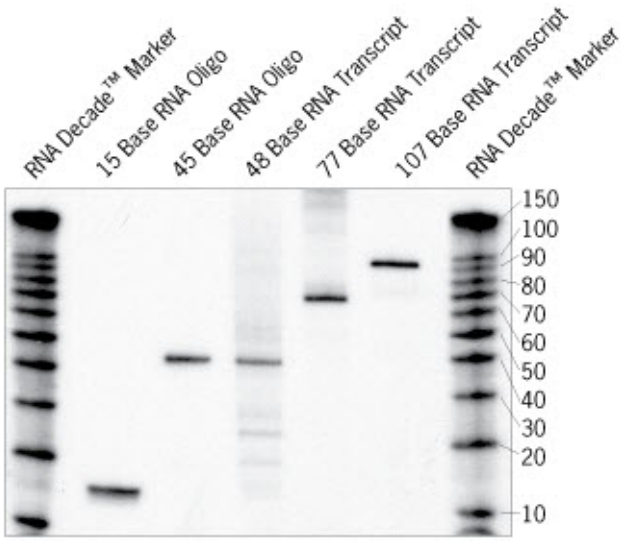

Figure 1 Decade™ Markers. Decade™ Markers (25 ng) were end-labeled and cleaved as described in the accompanying protocol. RNA oligonucleotides of 15 and 45 bases and three transcripts of 48, 77, and 107 bases were also end-labeled and assessed on a 10% polyacrylamide/8 M urea gel. The gel was exposed to film for 5 minutes using an intensifying screen at –80°C.

Handling instructions

RNA is very sensitive to degradation by exogenous ribonucleases introduced during handling. Wear gloves when handling this product. Use RNase-free reagents, tubes, and barrier pipette tips.

Thawing instructions

Thaw just to completion at 37°C, vortex for a few seconds when fully thawed, and place on ice. Aliquot the RNA, if necessary, to minimize freeze-thaw cycles (≤5).

Using Decade™ Markers

These molecular weight markers are ideal for gel analysis of small RNA molecules such as tRNAs, miRNAs, snRNAs, and snoRNAs. The Decade™ Markers are recommended for use with the *Silencer*® siRNA Construction Kit (Cat. no. AM1620), the MEGAshortscript™ T7 Kit (Cat. no. AM1354), and *mir*Vana™ products for miRNA analysis (see **www.lifetechnologies.com/mirna**).

Prepare Decade™ Markers

1. Mix the following in a nuclease-free tube.

Decade™ Marker RNA (100 ng)	1 µL
Nuclease-free Water	6 µL
10X Kinase Reaction Buffer	1 µL
[γ–^{32}P]ATP, ≥3,000 Ci/mmol	1 µL
T4 Polynucleotide Kinase	1 µL

2. Incubate at 37°C for 1 hour, then add:

Nuclease-free Water	8 µL
10X Cleavage Reagent	2 µL

3. Incubate at room temperature for 5 minutes, then add 20 µL of Gel Loading Buffer II.
4. Heat the mixture at 95°C for 5 minutes.
5. Load the Decade™ Markers on a denaturing polyacrylamide gel.

   Note: The appropriate loading volume will depend on the size of the wells being used, the age of the radioisotope, and the exposure time. Typically, 4 µL in a lane that is approximately 5 mm in width will give good signal after a 1 hour exposure.

Gel recommendations

A 10–20% polyacrylamide/7 M Urea gel gives the best resolution in the size range of the 10 Decade™ Markers; however, the Decade™ Markers can be used on any denaturing polyacrylamide gel (see Figure 1). Note that an uncut band will be visible at 150 nt.

Storage of labeled Decade™ Markers

Unused Decade™ Markers can be stored at –20°C. For subsequent use, thaw the Decade™ Markers, remove an appropriate volume, and heat for 5 minutes at 95°C before loading on the gel.

Quality control

Relevant kit components are tested in the following assay:

RNase activity: A sample is incubated with labeled RNA and analyzed by PAGE.

Functional testing: Tested functionally as described in this protocol.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.