RiboCut: User Guide

RNA Marker for Ribosome Profiling

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Introduction

The purification of ribosome-protected fragments (RPFs) represents one of the critical steps of the ribosome profiling (Ribo-Seq) workflow. RPFs purification is usually carried out via polyacrylamide gel electrophoresis, followed by the excision of fragments with length of ~30 nucleotides. Purification of RPFs has to be performed precisely, as inaccurate excisions can significantly increase the abundance of ribosomal RNAs (rRNA) and other unwanted contaminants.

The **RiboCut Marker** consists of three PAGE-purified RNA oligos (27, 29 and 32 nucleotides in length) (Fig. 1) with a phosphate at their 3' end, designed to facilitate the excision of RPFs from denaturing polyacrylamide gels. The RiboCut Marker is compatible with Ribo-Seq samples derived from different species (e.g. human, mouse, rat, *C.elegans*, *D. melanogaster*).

Catalogue Number: dp-M100-001
The siTOOLs Biotech RiboCut Marker is a mix of three 3'-phosphate RNA oligos (27, 29 and 32 residues long) designed to aid the precise excision of ribosome protected fragments (RPFs) during Ribo-Seq sample preparation. The RiboCut Marker is intended for use with TBE-Urea polyacrylamide gels and SYBR Gold Nucleic Acid Gel Stain.

Contents of RiboCut kit

Abbreviation and Volume

* Follow all handling instructions provided in the Safety Data Sheets (SDSs).

Catalogue	Description	Quantity	Storage
dp-M100-001	RNA Oligo Mix	1 tube (dry / 100 gel lanes)	-80°C
	2x Loading Buffer	1 tube (275 µl /100 gel lanes)	-20°C
	RNAase Free Water	1 tube (1lm)	-20°C

Wear appropriate protective eyewear, clothing and gloves. SDSs are available at: www.sitoolsbiotech.com/resources/safety-data-sheets

2x Loading Buffer: 90% de-ionized formamide, 10% TBE Buffer, 0.025% Bromophenol blue, 0.025% Xylene-Cyanol

Additionally Required Reagents and Materials

- RNase Free tubes
- 15% TBE-Urea Polyacrylamide Gels (e.g., EC6885BOX)
- SYBR Gold Nucleic Acid Gel Stain (Thermofisher S11494)

RiboCut Kit Protocol

1. Resuspension of RNA Oligo Mix

The RNA Oligo Mix is delivered dry and has to be resuspended prior to use.

a. Briefly spin down the tube (●) containing the RNA Oligo Mix.

b. Add 275 µl of RNAse-free water (●).

c. Close the tube and vortex well (20-30 seconds).

d. Briefly spin down tube with the resuspended RNA Oligo Mix.

e. To avoid multiple freeze-thaw cycles, it is recommended to aliquot the RNA Oligo Mix after resuspension.

f. Store aliquots at -80°C.

2. Prepare and Load the RiboCut RNA Marker

a. Thaw one aliquot of resuspended RNA Oligo Mix and vortex tube well.

b. Add 2.5 µl of resuspended RNA Oligo Mix and 2.5 µl of 2x Loading Dye (●) in a RNase-free tube.

c. Mix by pipetting up and down several times.

d. Briefly denature the mix for 2 min at 70°C.

e. Before loading the RNA marker, flush the gel-wells with TBE buffer using a syringe to remove the urea.

f. Load 5 µl of the mix.

g. After the run, stain the gel for 8-10 minutes with SYBR Gold in TBE buffer. Visualize RNA with a UV-Transilluminator and proceed with RPF band excision and gel extraction.

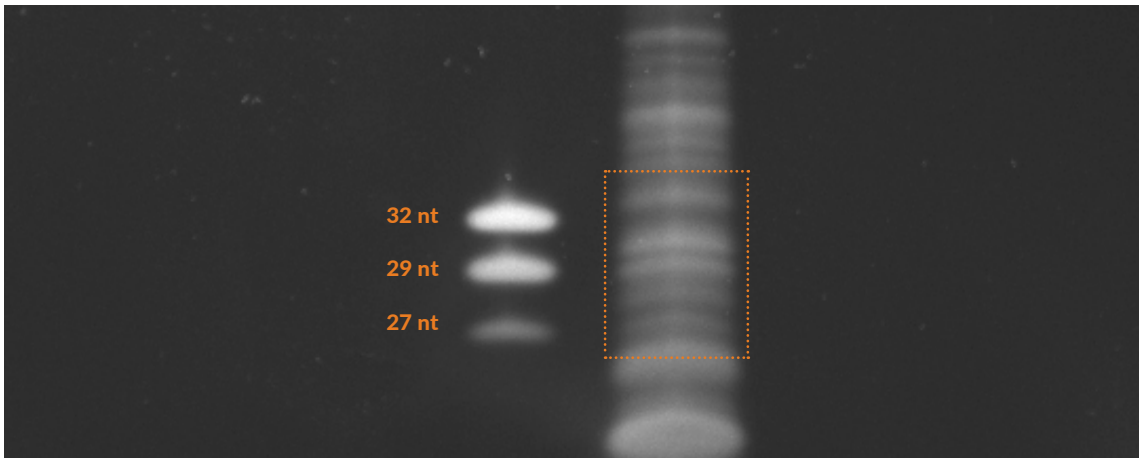

Figure 1 RiboCut Marker (left lane) and RNA digest (right line) on denaturing 15% polyacrylamide gel (180V, 60 min) stained with SYBR Gold (8 min). Orange box indicates gel area to be excised.

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