Enzymax LLC
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Product Information

Product Name: RNA Linker
Catalog #: RLK25
Size: 500pmol (25µl)
Order: info@enzymax.net
Tel: 859-219-8482
Fax: 859-219-0653
Web: www.enzymax.net

Product: RNA linker

Product Description:

RNA Linker is used in our RNA ligase-mediated (RLM) 5' RACE (Rapid Amplification of cDNA Ends) kit (Cat#KIT96) and RNA library cloning or preparation kit (Cat#KIT97).

Components:

25 µl RNA linker:
ACACUCUUUCCCUACACGA

RNA Linker usage in RNA RACE procedure:

RNase inhibitor may be needed if samples have RNase contamination. Use of RNA inhibitor is recommended.

Step 1: 5' decapping

~1 µg total RNA*	3.5 µl
RNA linker	1 µl
RNA decapping enzyme	0.5 µl
PEG-8000 (mix well in the reaction)	2.5 µl
10x ligation and decapping buffer (with ATP)	1 µl
$MnCl_2$	1 µl

Assemble the above components in a DNase/RNase free tube and incubate the tube at room temperature for 1 h, then inactivate at 65 °C for 15 min.

* RNA isolated by column, phenol, or Trizol-based methods are suitable for this kit. If RNA is partially degraded, it may be necessary to pre-treat sample with phosphatase (Calf Intestinal Phosphatase or CIP). RNA samples should be EDTA-free.

Step 2: 5' Ligation

Quick spin heat inactivated sample and add 0.5 µl 5' RNA Ligase and incubate at 16 °C for 2~3 hours, or 4 °C for overnight.

Step 3: Reverse transcription (RT)

Reverse primer (10 µM) **	0.5 µl
dNTP	1 µl
H_2O	4 µl

Add above components into step 2; incubate at 65 °C for 15 min, then keep on ice for 2 min.

Add components below and incubate at 42 °C for 30 min.

RT dilution buffer	2 µl
DTT	1 µl
Reverse transcriptase	0.5 µl

This is a gene specific reverse primer, which is not included in the kit. However, it can be substituted by random hexamer. When using random hexamers (use 20 µM instead), incubate samples at room temperature for 10 min before RT. Contact us for **global 5'RACE kit.

Step 4: PCR and nested PCR:

NOTE: Except for the common primer, all other PCR reagents are not included in this kit. PCR reagents are sold separately. The following procedures are based on our tests and need to be optimized by customers.

1. PCR Reaction (1st PCR reaction): 50 µl

10xPCR buffer	5 µl
25 mM dNTP	0.6 µl
10 µM 5' common primer (outer primer)	1 µl
10 µM 3'gene specific primer (outer primer)	0.5 µl
RT product from step 2	1 µl
PCR polymerase	0.5 µl
H_2O	41.4 µl

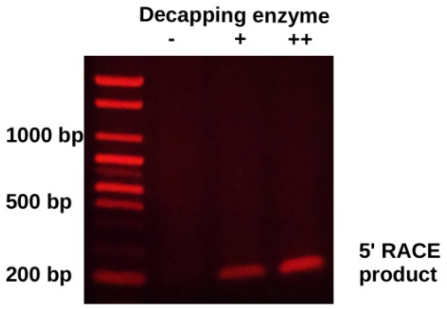
1 cycle	94°C 2 min
15 cycles	94°C 20 sec 54 °C 20 sec 68°C 1min
1 cycle	4°C

2. Nested PCR Reaction: 50 µl

10x PCR buffer	5 µl
25 mM dNTP	0.6 µl
10 µM 5' common primer (outer primer)	1 µl
10 µM 3' nested gene specific primer ***	1 µl
PCR product from 1st PCR reaction	1 µl
PCR polymerase	0.5 µl
H_2O	40.9 µl

***Also called inner primer which is designed at upstream of 3'gene specific primer (outer primer). Instead of provided 5'common primer, you can also design inner forward primer, which is at downstream of 5'common primer. Link for primer design:http://www.clcsupport.com/clcgenomicsworkbench/650/Nested_PCR.html.

1 cycle	94°C 2 min
24 cycles	94°C 20 sec 54 °C 20 sec 68°C 1min
1 cycle	4°C



5' RACE product form different amount of decapping enzyme