Enzymax LLC
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Product Information

Product Name: 5' RNA Ligation Kit
Catalog #: LK25
Size: 500 U (25 µl)

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Product: 5' RNA Ligation Kit

Product Description:

5' RNA ligation kit is optimized for using in RNA ligase-mediated (RLM) 5' RACE (Rapid Amplification of cDNA Ends) and RNA library cloning or preparation for sequencing. It catalyzes the ligation between RNA linker (with 3'-hydroxyl termini) and input RNA (with 5'-phosphate). Below is the description for enzyme usage in decapping/ligation step from our RNA RACE kit (KIT96).

Components:

30 µl 10x ligation and decapping buffer (with ATP)
25 µl 5' RNA ligase

Enzyme 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 and ligation

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

Assemble the above components in a DNase/RNase free tube and incubate the tube at room temperature for 2-8 h.

* 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: 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 3: 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

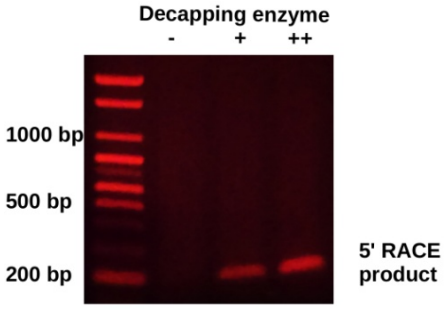
1 cycle	94°C 2 min
15 cycles	94°C 20 sec 54 °C 20 sec 68°C 1min
1 cycle	4°C

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 products with different amounts of decapping enzyme