Enzymax LLC
870 Corporate Drive, Suite 201; Tel: (859) 219-8482, Fax: (859) 219-0653; Web: www.enzymax.net

Product Information

Product Name: RNA 5' RACE KIT
Catalog #: KIT96
Size: 20 Reactions

Order: info@enzymax.net
Tel: 859-219-8482
Fax: 859-219-0653
Web: www.enzymax.net

Product: 5' RNA RACE Kit

Product Description:

This kit is for RNA ligase-mediated (RLM) 5' RACE (Rapid Amplification of cDNA Ends). It is used to clone the 5' end of capped RNA transcribed by Pol II. The procedure is one-tube without buffer change between reactions, and it is convenient, fast and sensitive.

Components:

30 µl 20x ligation and decapping buffer (without ATP)
75 µl 50% PEG-8000
20 µl RNA ligase
20 µl RNA decapping enzyme
20 µl Reverse transcriptase
25 µl 20 µM RNA linker: ACACUCUUUCCCUACACGACGCUCUUCC GAUCU
60 µl 10 µM 5' common primer (outer forward primer): ACACTCTTTCCCTACACGA
30 µl 10 mM dNTP
30 µl 100 mM DTT
60 µl 10x RT dilution buffer
12 µl 20 mM ATP

Procedure:

RNA inhibitor is not recommended unless the samples have RNase contamination. PEG should be kept at room temperature and is added using 20 µl tips (mix well by pipetting).

Step 1: 5' decapping and ligation (**20**µl reaction volume)

H_2O	X µl
1-2 µg total RNA*	X µl
20x ligation and decapping buffer (without ATP)	1 µl
PEG-8000 (mix well in the reaction)	3 µl
RNA linker	1 µl
ATP	0.5 µl
RNA decapping enzyme	1 µl
RNA ligase	1 µl

Assemble the above components in a DNase/RNase free tube and incubate the tube at room temperature for 3-4 h (samples can be kept 4 °C overnight after the incubation), then inactivate at 65 °C for 15 min.

*1-2 µl heat-sensitive RNA phosphatase (Cat#97) can be added with the first 3 components, incubated for 30 min and heat-inactivated at 95 °C for 5 min. Then add the rest of the components for incubation. However, this step is not necessary unless for making a high-throughput sequencing library with random priming.

* 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 (FastAP Thermosensitive Alkaline Phosphatase is recommended). RNA samples should be EDTA-free.

Step 2: Reverse transcription (RT)

Reverse primer (10 µM) **	0.5 µl
dNTP	1 µl
RT dilution buffer	2 µl

Add above components into step 1; 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.

DTT	1 µl
Reverse transcriptase	1 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. To increase PCR specificity, add 5% DMSO or special PCR enhancing regents (sold in 3'RACE kit).

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

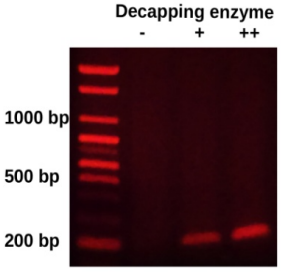
1 cycle	94°C 2 min
15 cycles	94°C 20 sec 54 °C 20 sec 68°C 1min
1 cycle	4°C

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