Product Information

Enzymax, LLC

Product Name: EZdeCAP
Catalog #: 96
Size: 250 pmol/250 U (45U/µl) *Dilute before use

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Product: EZdeCAP decapping enzyme

Product Description:

Decapping is a critical step in eukaryotic mRNA turnover. This is an enzyme mixture specifically hydrolyzes methylated capped RNA (m7 GTP caps) to release m7GDP and5′ monophosphorylated RNA.

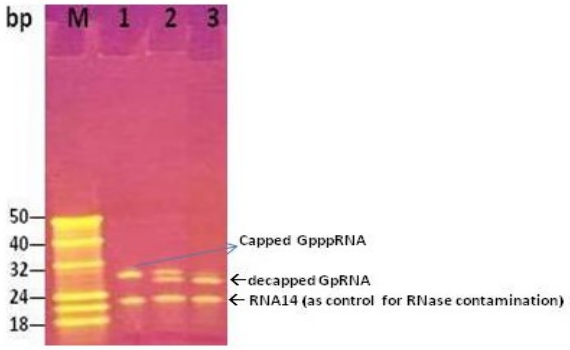
Unit definition: 1U is the amount to decap 1 pmol of capped RNA substrate at 37°C for 30 minutes.

Tested in following applications:

Decapping RNA (>22bp):

Usage: 2.5pmol (2.5U) for 2.5pmol small RNA
Reaction Volume& time: 10µl, incubation at 37°C for 1 hour
Recommendation: Add RNase inhibitor to the final concentration of 0.5-1U/µl in the reaction.

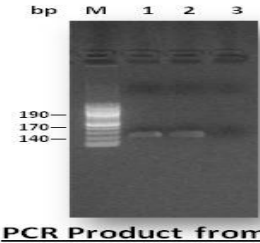
Gel for decapping efficiency:



5'RACE:

Usage: 10-20 pmol (10-20 U) for1µg total RNA
Reaction Volume: 40µl
Procedure:

1. Decapping: use 10-20 pmol enzyme for 1µg total RNA, 37°C for 1 hour.
2. Purified by Tini RNA column* or phenol extraction followed by ethanol precipitation
3. 5' ligation (10µl): 16°C for overnight.
4. RT: 42°C for 50 min (add primer for annealing at 65°C, 15 min before RT)
5. PCR and nested PCR (50 µl)
6. Run PCR gel: show PCR amplification with 10 and 20pmol decapping enzyme.



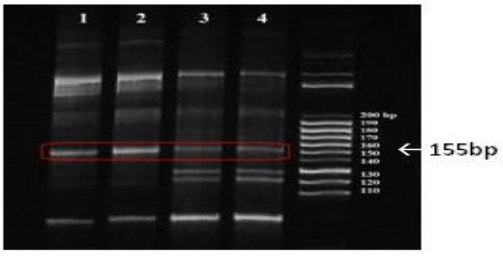
PCR Product from 5'RACE
Lane M: Size marker
Lane 1: with 10pmol EZdeCAP enzyme
Lane 2: with 20pmol EZdeCAP enzyme
Lane 3: without EZdeCAP enzyme

Small RNA Cloning:

Usage: 2.5 pmol (2.5 U) for 6 µg small RNA

Procedure:

1. Small RNA (18-30nt) purified from 6 µg total RNA
2. CIP at 50°C for 1 hour
3. Purified by Tini RNA column* or phenol extraction followed by ethanol precipitation
4. Decapping: use 2.5 pmol in 10-15ul reaction volume. Purified by Tini RNA column* or phenol extraction followed by ethanol precipitation
5. 3' ligation: 16°C for 2 hours.
6. Annealing: 65°C for 15 min.
7. 5' ligation: 16°C for 3 hours.
8. RT: 42°C for 20 min.
9. PCR: in 50 µl volume
10. Run gel (6% PolyAcrylamide) to check PCR products: show correct PCR products at range of 145-170bp.
11. Recover PCR product with correct size from gel.
12. Samples are ready for sequencing.



PCR Amplification: 8% Native PAGE gel with EtBr staining
Lane 1: Cloning small RNA using 1 ug total RNA without purification
Lane 2: Cloning small RNA using purified smRNA from 1 µg total RNA
Lane3: Cloning small RNA using total RNA from single worm (~20ng)
Lane4: Cloning small RNA using purified small RNA from single worm.

*Tini RNA spin column is designed and sold in bulk: $49 for 50 columns (compatible with all commercially available RNA kits with silica membrane based spin column. So, it can be used with all the solutions in the kits)
http://www.enzymax.net/columns/tini_spin_RNA_column.htm

Decapping Reaction Buffers: 1X

10 mM Tris-HCl at pH 7.5
100 mM NaCl
2 mM $MgCl_2$
1mM DTT
1mM $MnCl_2$

10x decapping Reaction Buffer (w/o $MnCl_2$):

Included

$MnCl_2$ (50mM): Included. Add it to the decapping reaction mixture to the final concentration of 1mM to enhance the decapping activity. It is **Optional**!
Note: The reaction buffer with $MnCl_2$ may turn into light yellow during the storage, it is **OK**, just mix and use as it is.

Enzyme Dilution Buffer: Not Included
10 mM Tris-HCl at pH 7.5, 100 mM NaCl, 10% Glycerol

NOTE: Add RNase inhibitor to the final concentration of 0.5-1U/µl in the reaction.

Storage: Stable for 12 months at -80°C from date of shipment. Please aliquot to avoid repeated freezing and thawing.