Enzymax, LLC

Product Information

Product Name: Tobacco dcapping enzyme
Catalog #: 87
Size: 255 pmol / 255U
Concentration: 15-40pmol/µl (15-40 U/µl)

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Product: Tobacco decapping enzyme

Product Description or Background:

Tobacco decapping enzyme catalyzes decapping of mRNA, which is a critical step in eukaryotic mRNA turnover. This enzyme hydrolyzes methylated capped RNA (m7 GTP caps) to release m7GDP and5′ monophosphorylated mRNA.

Source: Tobacco enzyme

Molecular Weight:

Full length Tobacco decapping enzyme (~ 40 kDa)

Presentation: Purified Tobacco decapping enzyme is supplied at a concentration range from 15-40 µM in a buffer containing 50 mM-Tris (pH 8.0), 500 mM NaCl and 10% glycerol.

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

Decapping Reaction Buffers: 1X
10 mM Tris-HCl, pH 7.5
100 mM NaCl
2 mM $MgCl_2$
1 mM DTT

Enzyme dilution buffer: Not included
10 mM Tris-HCl, pH 7.5
100 mM NaCl
10% Glycerol

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

$MnCl_2$ (50 mM): Included. Add it to the decapping reaction mixture to the final concentration of 1 mM to enhance the decapping activity.
Note: The reaction buffer with $MnCl_2$ may turn light yellow during storage, which is still usable.

Recommended for decapping reaction:
Add RNase inhibitor to the final concentration of 0.5 -1 unit/µl.

Contaminating Activity Assay: The decapping enzyme is free of detectable RNase activity when 3 pmol of small RNA (23 nt) was incubated with 5 pmol of the enzyme for 1 hour at 37°C (Figure 2, the RNA14 band).

SDS-PAGE gel picture:

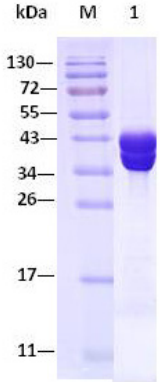
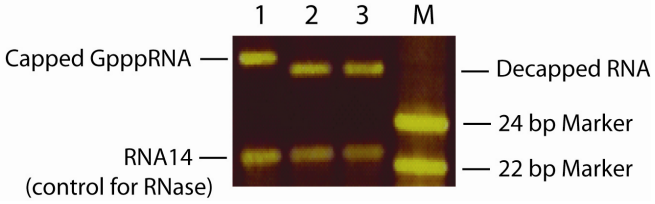


Figure 1. Purified Tobacco decapping enzyme: Tobacco decapping enzyme (800 ng) was analyzed by electrophoresis on a 15% SDS- polyacrylamide gel and visualized by staining with Coomassie blue. Protein size markers are indicated on the left.

Decapping Assay (Figure 2):
Lane M: RNA Marker
Lane 1: 2.5 pmol substrates without enzyme
Lane 2: 2.5 pmol substrates with 2.5 pmol enzyme
Lane 3: 2.5 pmol substrates with 5 pmol enzyme
Note: Substrates are capped GpppRNA and uncapped RNA14 (used as control for RNase contamination)



Suggested condition for used in 5' RACE:
Reaction temperature and time: 37°C, 60 min
Reaction volume: 40 µl
RNA substrates: 1 µg
10X buffer with $MnCl_2$: 4 µl
Decapping enzyme: 8-15 U (8-15 pmol) 1 µl
RNase inhibitor: 20 U
ddH_2O: add to final volume of 40 µl

NOTE: The optimal reaction condition may be different for other applications.

Suggested condition for micro RNA cloning:
Usage: 5 pmol (5 U) for 6 µg small RNA