Tinzyme Co., Limited

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T4 RNA Ligase 2, truncated KQ

Product Number: LG09

Shipping and Storage

Stored at -20°C, valid for two years.

Component

Component	20KU
T4 RNA Ligase 2, truncated KQ (200 U/μL)	100μL
10× T4 Rnl2 KQ Buffer	1mL
50% PEG 8000 (RNase-free)	1mL

Description

T4 RNA Ligase 2, truncated KQ is a double-point mutant (R55K, K227Q) of truncated T4 RNA Ligase 2. While maintaining ligation efficiency, it can greatly reduce non-specific ligation of RNA (RNA concatemerization or self-circularization). This enzyme specifically ligates 5'-preadenylated DNA or RNA to the 3'-OH end of RNA, and is highly suitable for ligating adapters to small RNAs for cDNA library construction. Ligation with this enzyme does not require ATP, but requires 5'-preadenylated adapters.

Unit Definition

200 units (U) is defined as the amount of enzyme required to ligate 80% of 31-mer RNA to the end of preadenylated 17-mer DNA within 1 hour at 25°C.

Inactivation Condition

65°C for 20 min.

Quality Control

1. Protein Purity: Protein purity is no less than 95% as determined by SDS-PAGE gel electrophoresis.
2. Endonuclease Activity: After incubating 200 U of T4 RNA Ligase 2, truncated KQ with 200ng of supercoiled plasmid DNA in a 20μL T4 Rnl2 KQ Buffer reaction system at 37°C for 4 hours, less than 10% of the plasmid DNA is converted to nicked or linear form as detected by agarose gel electrophoresis.
3. DNase Activity: After incubating 200U of T4 RNA Ligase 2, truncated KQ with 15ng of double-stranded DNA fragment in a 20μL T4 Rnl2 KQ Buffer reaction system at 37°C for 16 hours, no change is observed in the double-stranded DNA fragment as detected by agarose gel electrophoresis.
4. RNase Activity: After incubating 200U of T4 RNA Ligase 2, truncated KQ with 500ng of RNA in a 10μL T4 Rnl2 KQ Buffer reaction system at 37°C for 1 hour, no less than 90% of the RNA remains intact as detected by agarose gel electrophoresis.
5. Phosphatase Activity: Add 200U of this enzyme and 2.5mM p-Nitrophenyl Phosphate (pNPP) into a 200μL reaction system, incubate at 37°C for 4 hours; alkaline phosphatase activity is <0.0001 U.
6. Host DNA Residue: Determined by the quantitative PCR method (the third method) in General Rule 3407, Volume IV of the Chinese Pharmacopoeia 2020 Edition; the residual amount of E. coli host cell DNA in this product is <10 copies/200U.

Protocol

1. Prepare the following reaction system on ice:

Component	Suggested Volume	Final Concentration
10× T4 Rnl2 KQ Buffer	2μL	1×
T4 RNA Ligase 2, truncated KQ (200 U/μL)	1μL	10U/μL

50% PEG 8000 (RNase-free)	4μL	10%
RNA Substrate (10μM)	2μL	1μM
5'-Preadenylated Adapter (20μM)	2μL	2μM
RNase Inhibitor (40 U/μL)	1μL	2U/μL
Nuclease-Free Water	up to 20μL	-

2. Mix thoroughly and centrifuge briefly, then incubate at 25°C for 1~2 hours.
3. After the reaction, add Proteinase K or EDTA to the product to terminate the reaction.

Note

1. When using this product to ligate RNA with 5'-preadenylated adapters, the recommended concentration of PEG 8000 is 10%~30%, which helps to improve ligation efficiency.
2. To prevent RNase contamination, RNase inhibitor can be added. Wear clean gloves and mask during operation; all consumables such as pipette tips and centrifuge tubes used in the experiment shall be RNase-free.
3. For your safety and health, please wear a lab coat, disposable gloves and mask during experimental operation.