TECHNICAL SHEET

PRODUCT NAME: **Reverse Transcriptase (RT2) with glycerol, 250000 U**

Code:	MT-25RT2G
Physical State:	Liquid
Source:	*Escherichia coli.*
Description:	Reverse Transcriptase recombinant protein expressed and purified from *Escherichia coli.*
Appearance:	Clear
Purity:	>95%
Activity:	100 U/µL
Storage Conditions:	Storage at -20 or -80ºC.
Health & Safety Information:	Good Laboratory Practices should be followed when handling this material. The end user assumes all responsibility for care, custody and control of the material, including its disposal, in accordance with the respective national regulations.
Presentation:	50 % glycerol, HEPES Buffer with additives and salts, pH: 7.4.

THIS PRODUCT IS INTENDED FOR RESEARCH USE ONLY.

Date: 17/08/2022

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General description

Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase RNase H minus (MT-25RT2) is a highly sensitive and stable enzyme. It is an RNA-dependent DNA polymerase that can be used to generate first- strand cDNA from polyA mRNA, total RNA or viral RNA for use in downstream applications such as RT- (q)PCR or cDNA cloning. The enzyme is isolated from a strain of Escherichia coli strain carrying the Moloney-Murine Leukemia Virus Reverse Transcriptase gene with different point mutations in the RNase H domain, which increases the thermostability of the enzyme and support greater cDNA yield of full-length transcripts (>5Kb) than wild type M-MLV Reverse Transcriptase. This enzyme has a molecular weight of approximately 76kDa, and its optimum extension temperature is 50 ºC. The presence of glycerol makes this product perfect to be used in developing non-lyophilized molecular biology products.

Storage and stability

It is recommended to keep it at -80ºC for optimum stability. Repeated freeze/thaw should be avoided. When stored under these conditions the polymerase retained full activity until the expiry date.

Quality control

Each lot of M-MMLV Reverse Trancriptase RNase H minus is tested sensitivity. Purity is also checked by SDS-electrophoresis and HPLC.

Analytical sensitivity Assay

Analytical sensitivity of each lot of RT2 Reverse Transcriptase is evaluated performing standard curves in parallel with a reference lot. 10-fold serial dilution of control RNA is performed and 5 µl of each dilution are added to 20 µl reaction mixtures containing RT2 Reverse Transcriptase (100 U), TAQ DNA polymerase (10 U), specific primers and probe (500 nM and 250 nM respectively), 10 X Reaction Buffer (1 X), MgCl2 (3 mM) and dNTPs (0.8 mM each). Amplification conditions are those specify for Taq DNA Polymerase. Direct detection of PCR products is monitored by measuring the relative fluorescence units (RFU) produced by the result of probe hydrolysis after every cycle. And the resulting parallel standard curves are compared and assessed by analyzing the fluorescence, the minimum concentration of nucleic acids detection, Ct values and the sigmoid shape of the curves.

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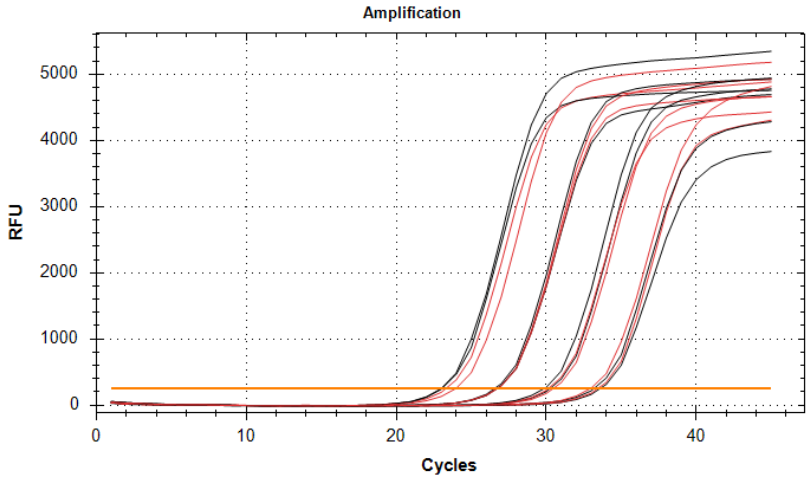
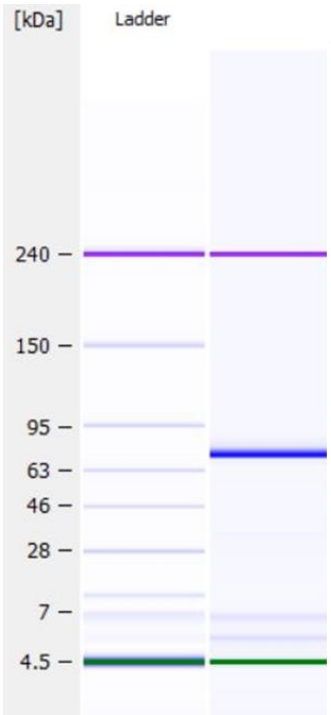


Image: comparation of parallel stand curves of a reference lot of RT2 Reverse Transcriptase (in black) and an evaluation lot of RT2 Reverse Transcriptase (in red). Similar efficiency of amplification is observed.

SDS-electrophoresis

SDS-PAGE gel in reducing conditions:



If technical support is needed, please contact us (bioscience@certest.es).

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