TECHNICAL SHEET

PRODUCT NAME: Kit Reverse Transcriptase (RT2), lyophilized (x50000 U)

Code:	MT-E27RT2
Physical State:	Lyophilized
Source:	*Escherichia coli.*
Description:	Reverse Transcriptase recombinant protein expressed and purified from *Escherichia coli.*
Appearance:	Powder
Purity:	>95%
Storage Conditions:	Storage at room temperature.
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:	HEPES Buffer with additives and salts, pH: 7.4.

THIS PRODUCT IS INTENDED FOR RESEARCH USE ONLY.

Date: 17/08/2022

Pol. Industrial Río Gállego II, Calle J, Nº 1,
50840, San Mateo de Gállego,
Zaragoza (SPAIN)
Tel. +34 976520354
certest@certest.es
www.certest.es

General description

This kit contains 50000 U of lyophilized Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase RNase H minus (MT-25RT2). Certest RT2 Reverse Transcriptase 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 increase 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 50ºC.

Package content

Reference	Description	Vials
MT-27RT2	Reverse Transcriptase (RT2), lyophilized (x50000 U)	1 x vial (clear)
MT-36REC	1X Reconstitution Buffer	0.5 mL; 1 x vial (clear)
MT-36MGCL	100 mM MgCl2 Buffer	1.5 mL; 1 x vial (blue)
MT-36RT2	10X RT2 Reaction Buffer	1.5 mL; 1 x vial (purple)

Storage

The kit is shipped at room temperature. Store at room temperature or below (until expiry date, see product label). Reconstituted RT2 must be stored at -20°C. Reconstitution instructions for the lyophilized enzyme are as it follows.

1) Centrifuge the vial of lyophilized enzyme at 12000 x g for 20 seconds.
2) For each tube containing 50000 U RT2 Reverse Transcriptase lyophilized, add 250µL of 1X Reconstitution Buffer to reconstitute 200U/µL enzyme solution. Gently pipette up and down to dissolve the pellet/powder.
3) Place on ice, and aliquot into smaller volumes. Repeated freezing and thawing should be avoided.
4) For long-term storage, store at -20°C.

Quality control

Each lot RT2 Reverse Transcriptase is tested for sensitivity Purity is also checked by SDS-electrophoresis and HPLC.

Pol. Industrial Río Gállego II, Calle J, Nº 1,
50840, San Mateo de Gállego,
Zaragoza (SPAIN)
Tel. +34 976520354
certest@certest.es
www.certest.es

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.

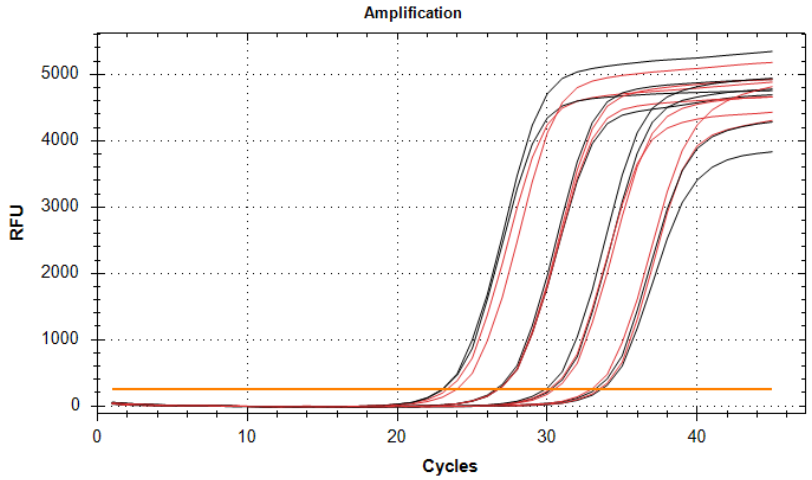
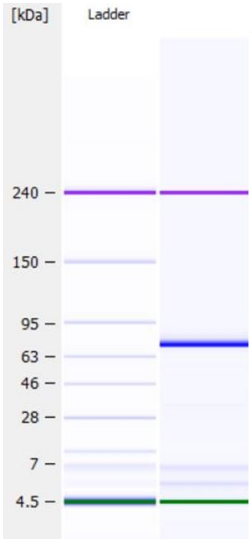


Image: comparation of parallel stand curves of a reference lot of RT2 Reverse Transcriptase (in black) and an evaluation lot of RT2 Reverse Transcriptase (in red). Similar efficiency of amplification is observed.

SDS-electrophoresis

SDS-PAGE gel in reducing conditions:



Pol. Industrial Río Gállego II, Calle J, Nº 1,
50840, San Mateo de Gállego,
Zaragoza (SPAIN)
Tel. +34 976520354
certest@certest.es
www.certest.es

Usage instructions

1) RNA denaturation and primer annealing. RNA is incubated with the primer and heated to 65-70°C for 5 minutes. Then incubated on ice for 1 minute. Note: see table below.

2) DNA polymerization. Prepare the following mix on ice and mix gently by pipetting. Then, incubate the mix at 45ºC for 30 min.

Component	Volume	Final concentration (recommended)
10X RT2 Reaction Buffer	2 µL	1X
Primer: Oligo dT Random Primer Gene-specific primer	variable	 1-2 µg Oligo dT 2-5 µg Random Primer 1-2 µg Gene-specific primer
dNTPs Mix	variable	2 mM (0.5 mM of each)
100 mM $MgCl_2$ Buffer	variable	1.5 mM-5mM
RT2 Reverse Transcriptase	0.5 µL	100 U
RNA template	variable	Total RNA (up to 5 µg) or mRNA (up to 0,5 µg)
Nuclease free water	Adjust to 20 µL	

3) Heat inactivation. Inactivate the reverse transcriptase by incubating the mix at 95ºC for 2 min, then chill on ice.

4) Use cDNA in downstream applications, direct cDNA analysis by gel electrophoresis or microarray, two step RT-PCR, or store at -20 ºC.

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

Pol. Industrial Río Gállego II, Calle J, Nº 1,
50840, San Mateo de Gállego,
Zaragoza (SPAIN)
Tel. +34 976520354
certest@certest.es
www.certest.es