Thermo SCIENTIFIC

PRODUCT INFORMATION

Phi6 RNA Replicase

#F-611L 300 U

Lot _ Expiry Date _

Ordering information

Component	#F-611S 60 U	#F-611L 300 U
Phi6 RNA Replicase, 1 U/µL	60 µL	300 µL
10X RNA Replicase Buffer	1.5 mL	1.5 mL
50 mM $MnCl_2$	500 µL	500 µL

Store at -20°C

37

www.thermoscientific.com/onebio

Description

The Thermo Scientific Phi6 RNA Replicase is a modified version of the protein P2 from bacteriophage Φ6. This RNA-dependent RNA polymerase catalyzes the synthesis of a full-length complementary RNA strand initiating from the 3' terminus of a single-stranded RNA. The polymerase does not require any oligonucleotide primer for the initiation. Due to a unique modification, the Phi6 RNA Replicase displays relatively low template specificity and is therefore capable of replicating a wide variety of RNA templates, as well as denatured DNA which contains a recognition sequence for the Phi6 RNA Replicase at it's 3' terminus.

Applications

Replication of ssRNA to dsRNA form.

Rev.2 53

Protocol

1. Set up a dsRNA synthesis reaction using the following reaction conditions:
   1X RNA Replicase Buffer
   1.5 mM $MnCl_2$
   20–100 ng/µL ssRNA
   0.1–0.2 mM ATP, CTP, UTP
   0.3–0.6 mM GTP
2. Add 1 U Phi6 RNA Replicase per 40 µL reaction volume.
3. Incubate at 32°C for 1–4 h.
4. Purify the amplified dsRNA using standard methods if necessary for your downstream application.

Component specifications

Phi6 RNA Replicase is purified from an *E. coli* strain that carries the modified P2 gene from bacteriophage Φ6.

Storage buffer: 50 mM Tris-HCl (pH 8.0 at 25°C), 0.1 mM EDTA, 100 M NaCl, 0.1% Triton® X-100 and 50% glycerol.

Reaction buffer: Phi6 RNA Replicase is supplied with 10X RNA Replicase Buffer and 50 mM $MnCl_2$ solution. 1X buffer contains: 50 mM Tris-acetate (pH 8.75 at 21°C), 50 mM NH_4Ac.

Unit definition: One unit is defined as the amount of enzyme that incorporates 1 nmole of UTP into acid-insoluble form at 32°C in 20 minutes in the following reaction mixture: 50 mM Tris-acetate (pH 8.75 at 21°C), 50 mM NH_4Ac, 1.5 mM $MnCl_2$, 10 % DMSO, 1 mM UTP; 1 µg poly (rA) and 1 µCi ^{3}H-UTP per 30 µL reaction volume.

Exonuclease assay: Incubation of 1 U of Phi6 RNA Replicase (4 h, 37°C, 50 µL) with 1 µg of sonicated [^{3}H]-DNA (3 × 105 cpm/µg) in the assay buffer released <0.5% of radioactivity.

Endonuclease assay: Incubation of 1 U of Phi6 RNA Replicase (2 h, 37°C, 50 µL) with 1 µg of ΦX174 RFI DNA in the assay buffer gave <10% conversion to RFII form.

Ribonuclease assay: Incubation of 1 U of Phi6 RNA Replicase (1 h, 37°C, 50 µL) with 1 µg of single-stranded MS2 RNA resulted in the similar RNA pattern as that produced without the enzyme.

Shipping and storage

Phi6 RNA Replicase is shipped on gel ice. Upon arrival, store the components at -20°C. Phi6 RNA Replicase is stable for one year from the assay date when stored and handled properly.

TECHNICAL SUPPORT
EMEA: ts.molbio.eu@thermofisher.com
Americas & APAC: ts.molbio@thermofisher.com

PRODUCT USE LIMITATION
This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.