Phi6 RNA Replicase

F-611S, 60 U
F-611L, 300 U

Store at -20°C.

Thermo SCIENTIFIC

1. Description

Phi6 RNA Replicase is a modified version of the protein P2 from bacteriophage Φ6. The RNA-dependent RNA polymerase catalyzes the synthesis of a full-length complementary RNA strand initiating from the 3' terminus of a single-stranded RNA. Due to the modification, Phi6 RNA Replicase displays relatively low template specificity and it is therefore capable of replicating a great variety of RNA templates, as well as denatured DNA that contains a recognition sequence for Phi6 RNA Replicase at its 3' terminus.

2. Package information

F-611S	60 U Material provided: Phi6 RNA Replicase 60 U (1 U/µl) 10x RNA Replicase Buffer 1.5 ml 50 mM $MnCl_2$ 500 µl
F-611L	300 U Material provided: Phi6 RNA Replicase 300 U (1 U/µl) 10x RNA Replicase Buffer 1.5 ml 50 mM $MnCl_2$ 500 µl

Material safety datasheet (MSDS) is available at www.thermoscientific.com/fzmsds.

3. Instructions

3.1 Synthesis and amplification of microgram quantities of dsRNA from dsDNA template

Phi6 RNA Replicase replicates denatured DNA strands using ribonucleotides to form double-stranded DNA-RNA hybrid molecules. Subsequently, Phi6 RNA Replicase displaces the DNA strand from the hybrid duplex while creating a double-stranded RNA molecule. The displaced ssDNA molecule can then serve again as a template molecule in a new amplification cycle.

3.2 Protocol

1. Prepare the desired dsDNA template by PCR amplification. Use primers that contain 18–22 nt template-specific sequence and an additional tail sequence at the 5' end:
   5' GGAAAAAAA-$N_{(18–22)}$ 3'
   $N_{(18–22)}$: the template-specific stretch in the primer
2. Set up a dsRNA synthesis reaction using the following reaction conditions:
   1x RNA Replicase Buffer
   1.5 mM $MnCl_2$
   20–100 ng/µl PCR-amplified template DNA
   0.1–0.2 mM ATP, CTP, UTP
   0.3–0.6 mM GTP
3. Denature template DNA by incubating the reaction mixture at 95°C for 2 min. Snap cool on ice.
4. Add 1 U Phi6 RNA Replicase per 40 µl reaction volume.
5. Incubate at 32°C for 1–4 h.
6. Purify the amplified dsRNA using standard methods if necessary for your downstream application.

4. 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 inutes 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 x 10^5 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

US: techservice.genomics@thermofisher.com
Europe, Asia, Rest of World:
techservice.emea.genomics@thermofisher.com

Web: www.thermoscientific.com/finnzymes

Product use limitation
This product has been developed and is sold exclusively for research purposes and in vitro use only. This product has not been tested for use in diagnostics or drug development, nor are they suitable for administration to humans or animals.

The quality system of Finnzymes Oy, a Thermo Fisher Scientific company, is certified according to standard

SFS-EN ISO9001:2008.

Version 1.0_07.2011

Thermo SCIENTIFIC

Part of Thermo Fisher Scientific