

Phi6 RNA Replicase

Product codes:

F-611S, 60 U

F-611L, 300 U

Stable for one year from the assay date. Store at -20°C.

1. Description

Phi6 RNA Replicase is a modified version of the protein P2 from bacteriophage $\Phi 6$. 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/ μ l) 10x RNA Replicase Buffer 1.5 ml 50 mM MnCl ₂ 500 μ l
F-611L	300 U Material provided: Phi6 RNA Replicase 300 U (1 U/ μ l) 10x RNA Replicase Buffer 1.5 ml 50 mM MnCl ₂ 500 μ l

Material safety datasheet (MSDS) is available at www.finnzymes.com.

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

- 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:
 $5' \text{ GGAAAAAAAA-N}_{(18-22)} 3'$
 $\text{N}_{(18-22)}$: the template-specific stretch in the primer
- Set up a dsRNA synthesis reaction using the following reaction conditions:
 1x RNA Replicase Buffer
 1.5 mM MnCl₂
 20–100 ng/ μ l PCR-amplified template DNA
 0.1–0.2 mM ATP, CTP, UTP
 0.3–0.6 mM GTP
- Denature template DNA by incubating the reaction mixture at 95°C for 2 min. Snap cool on ice.
- Add 1 U Phi6 RNA Replicase per 40 μ l reaction volume.
- Incubate at 32°C for 1–4 h.
- 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 $\Phi 6$.

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

Reaction buffer: Phi6 RNA Replicase is supplied with 10x RNA Replicase Buffer and 50 mM MnCl₂ solution. 1x buffer contains: 50 mM Tris-acetate (pH 8.75 at 21°C), 50 mM NH₄Ac.

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 NH₄Ac, 1.5 mM MnCl₂, 10 % DMSO, 1 mM UTP; 1 μ g poly (rA) and 1 μ Ci ³H-UTP per 30 μ l reaction volume.

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

Endonuclease assay: Incubation of 1 U of Phi6 RNA Replicase (2 h, 37°C, 50 μ l) with 1 μ g of Φ X174 RFI DNA in the assay buffer gave <10 % conversion to RFI form.

Ribonuclease assay: Incubation of 1 U of Phi6 RNA Replicase (1 h, 37°C, 50 μ l) with 1 μ g 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.

Warranty

Finnzymes Oy warrants that its products will meet the specifications stated on the technical data section of the data sheets, and Finnzymes Oy agrees to replace the products free of charge if the products do not conform to the specifications. Notice for replacement must be given within 60 days of receipt. In consideration of the above commitments by Finnzymes Oy, the buyer agrees to and accepts the following conditions:

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