

Phi6 RNA Replicase

F-611S/L

60 U/300 U 1 U/μl

Store at -20°C

Stable for one year from the assay date.

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.

Source: *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 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-cetate (pH 8.75 at 21°C), 50 mM NH₄Ac, 1.5 mM MnCl₂, 10% DMSO, 1 mM UTP; 1 μg poly (rA) and 1 μCi ³H-UTP per 30 μl reaction volume.

Exonuclease assay: Incubation of 1 U of Phi6 RNA Replicase (4 hours, 37°C, 50 μl) with 1 μg of sonicated ³H-ssDNA (3X10⁵ 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 RF1 DNA in the assay buffer gave <5 % 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.

Synthesis and amplification of microgram quantities of dsRNA from dsDNA template

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

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:
 $5' \text{ GGAAAAAAAA-N}_{(18-22)} 3'$
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 MnCl₂
 - 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 40μl 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.