AdvancedSeq LLC
6654 Owens Drive
Pleasanton, CA 94588

High Quality Sanger Sequencing Reagents Provider
www.advancedseq.com
info@advancedseq.com

ADS™ Phi29 RCA DNA Amplification Kit User Manual

Product Catalog Number:

Catalog Number	Unit Size	Components
220100	100 reactions	500 units phi29 DNA polymerase 1.2 mL 2X amplification mix 1.2 mL lysis buffer
220500	500 reactions	2,500 units phi29 DNA polymerase 6 mL 2X amplification mix 12 mL lysis buffer

Product Description: Phi29 DNA polymerase is a DNA polymerase from *Bacillus subtilis* phage Phi29. The monomeric protein contains an N-terminal 3'- 5' proofreading exonuclease activity and C-terminal DNA polymerase activity. This polymerase has exceptional strand displacement and processive synthesis properties. This product is purified from an *E. coli* strain that carries the phi29 DNA polymerase gene.

The ADS Phi29 RCA DNA Amplification Kit makes it easy for users to amplify DNA through Phi29 DNA polymerase rolling circle amplification (RCA). All reagents except Phi29 DNA polymerase are included in the kit to reduce errors and possible contaminants in reagent handling. Lysis buffer is also included for lysis of bacterial cells when colony or cell culture is used as the source of DNA template.

Application: Phi29 DNA polymerase has multiple applications such as rolling circle amplification of DNA template for Sanger sequencing or next-generation sequencing (NGS), whole genome amplification (WGA), recombination-based cloning, *in situ* genotyping, shRNA cloning and library screening.

Storage: The Phi DNA polymerase in the buffer (20 mM Tis-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 0.5 mM dithiothreitol (DDT), 0.5% Tween 20, 0.5% NP40, 50% Glycerol), the 2X Amplification Buffer and Lysis Buffer should be stored at -20 °C.

Phi29 DNA Polymerase Quality Test

Test	Results
DNAse activity	Not detected (37 °C for 2 hours)
Host cell DNA contamination	Not detected
Colony rolling circle cycle	Passed
Sanger sequencing	Passed

Rolling Circle Amplification (RCA) Protocols:

RCA Protocol for plasmid (for instance, pGEM™-3ZF (+))

1. Prepare the amplification mix as follows: for n reactions, add (n+1)*10 of 2X Amplification Buffer and (n+1)*0.5 µl of Phi29 DNA polymerase.
2. In a PCR tube, mix 1 µL pGEM™-3ZF (+) DNA (0.1-1ng/µL) with 8.5 µL 1X TE in PCR tube
3. Incubate the diluted DNA at 95°C for 2 mins, then spin it down
4. Add 10.5 µL of the amplification mix and mix well
5. Briefly spin the tube and place it in a PCR instrument. Run the following protocol:

   30 °C for 4 hours; 60 °C for 10 minutes; 4 °C forever

RCA Protocol for Bacterial Colony or Cell Culture:

A. Colony DNA preparation

1. Pick up a single fresh colony using a sterile toothpick
2. Resuspend the colony in 20 µL Lysis Buffer in a PCR tube and mix by tapping the tube gently
3. Incubate it at 95°C for 5 minutes
4. Spin it down at 15,000 g for 2 minutes, discard the pellet, save the supernatant (colony DNA) for RCA below

B. RCA protocol for colony DNA

1. Add 1 µL sample supernatant from step A. 4 to a PCR tube containing 8.5 µL H_2O
2. Prepare the amplification mix as follows: for n reactions, add (n+1)*10 of 2X Amplification Buffer and (n+1)*0.5 µl of Phi29 DNA polymerase.
3. Add 10.5 µL of amplification mix to the PCR tube in step 1
4. Mix the reagents well and spin it down
5. Place the tube in a PCR instrument and run the following:

   30 °C for 4 hours; 60 °C for 10 minutes; 4 °C forever