Product Information	
Phi29 DNA Polymerase	
Part Number	P7020-HC-L
Concentration	100,000 U/mL
Unit Size	2,000 U
Storage Temperature	-25ºC to -15ºC
Lot Number	(Shipment Specific)
Reference Number	(Shipment Specific)

Product Description: Phi29 DNA Polymerase responsible for the replication of the Bacillus Subtilis phage Phi29 (1). The enzyme is a highly processive DNA polymerase (up to 70,000 base insertions per binding event) with a powerful strand displacement activity (2) and a 3' →5' proofreading exonuclease function (3).

Product Specifications					
P7020					
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Endonuclease**	**E. coli DNA Contamination**
Units Tested	**n/a**	**n/a**	**100**	**100**	**100**
Specification	>99%	83,333 U/mg	Functional	No Conversion	<10 copies

Source of Protein: A recombinant E. coli strain carrying the Phi29 DNA Polymerase gene from bacteriophage Phi29.
Unit Definition: 1 unit is defined as the amount of polymerase required to convert 0.5 pmol of dNTP into acid insoluble material in 10 minutes at 30°C.
Molecular weight: 66,713 Daltons

Quality Control Analysis:
Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X Phi29 DNA Polymerase reaction buffer and added to 50 µL reactions containing λ Hind III DNA, 1X Phi29 DNA Polymerase Reaction Buffer, 3H-dTTP, 0.2 µM dTTP and 200 µM dATP, dCTP, dGTP. Reactions were incubated 10 minutes at 30°C, plunged on ice, and analyzed using the method of Sambrook and Russell (Molecular Cloning, v3, 2001, pp. A8.25-A8.26).
Protein Concentration (OD_{280}) is determined by OD_{280} absorbance.
Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.
Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.
Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.
E.coli 16S rDNA Contamination is evaluated using 5 µL replicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

Supplied in: 10mM Tris-HCl, 100mM KCl, 1mM DTT, 0.1mM EDTA, 0.5% Tween 20, 0.5% NP-40, 50% glycerol pH 7.4 @ 25ºC.
Supplied with: 10X Phi29 DNA Polymerase Buffer (B7020): 500mM Tris-HCl, 40mM DTT, 100 mM $(NH_4)_2SO_4$, 100 mM $MgCl_2$ pH 7.5 @ 25ºC.

Limitations of Use
This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.

References:

1. Blanco, L. and Salas, M. (1984) Proc. Natl. Acad. Sci. USA, 81, 5325-5329.
2. Blanco, L. et al. (1989) J. Biol. Chem., 264, 8935-8940.
3. Garmendia, C. et al. (1992) J. Biol. Chem., 267, 2594-2599.

Multiple Displacement Amplification Protocol Using Phi29 DNA Polymerase

Multiple Displacement Amplification (MDA) is a method for isothermal whole-genome amplification (WGA) of small amounts of template DNA utilizing Phi29 DNA polymerase and random hexamer primers to generate microgram quantities of DNA useful for a variety of downstream applications. The high processivity and strong strand-displacement activity of Phi29 allow for cycles of primer extension at multiple sites and displacement of the newly synthesized DNA resulting in the generation of kilobase-sized DNA product at a constant temperature. The high fidelity of Phi29 polymerase contributes to accurate amplification of starting template.

Materials needed:

- Phi29 DNA Polymerase (100,000 U/ml), P7020-HC-L
- 10X Phi29 DNA Polymerase Buffer, B7020L
- 10 mM dNTP solution mix, 201900
- Low EDTA TE Buffer (10 mM Tris pH 8.0, 0.1 mM EDTA), user supplied
- 1 mM Random Hexamer, user supplied
    - Note: for best results the random hexamer should have phosphorothioate bonds at the 3' ultimate and penultimate bases to prevent degradation by the Phi29 polymerase proofreading activity.
- Water bath, heat block, or thermal cycler

Guidelines for Reaction Setup

Because of the high sensitivity of MDA and its ability to amplify any input DNA regardless of sequence it is important to use precautions when assembling reactions to avoid cross-contamination and to use high quality water for reaction assembly.

The following procedure should be used as a guideline for further optimization. Reaction conditions may need to be adjusted individually for best results.

Template Denaturation

Bring DNA sample to 10 µl using low EDTA TE buffer. For example, combine 1 µl of sample with 9 µl low EDTA TE buffer in a suitable reaction tube.

Heat at 95°C for 3 minutes, then cool on ice.

Reaction Assembly

Prepare a master mix by combine the following reagents:

Component	Volume for 50 µl Reaction	Final concentration
Nuclease-free water	Variable	-
10X Phi29 DNA Polymerase Buffer	5 µl	1X
10 mM dNTP mix	2 µl	0.4 mM each
1 mM Random Hexamer	2 µl	40 µM
Phi29 DNA Polymerase (100,000 U/ml)	0.8 µl	1,600 U/ml
Final volume	**40 µl**	

Note: If a different final reaction volume is required, scale all components proportionally.

Combine 40 µl master mix with 10 µl cooled denatured DNA template.

Vortex gently to mix contents, then briefly centrifuge to collect components at the bottom of the reaction tube.

Incubate reactions at 30°C in a water bath, heat block, or thermocycler. Reactions are typically complete in 2-4 hours, but reactions can be incubated up to 18 hours.

If desired, Phi29 can be heat-inactivated by incubating at 65°C for 10 minutes.