I. DESCRIPTION

GenScript High-Stability PCR Kit contains Green *Taq* DNA polymerase, 10X reaction buffer, and 10 mM dNTP, all specially formulated for increased stability using our proprietary technology. The kit can be stored at 4°C for over six months or at room temperature (25°C) for one month without significant loss of activity. The stabilizers in the buffers also increase the stability of *Taq* DNA polymerase at higher temperature (72°C), increasing the DNA yield of PCR amplifications of long DNA. This kit facilitates quick and easy PCR setup without any need for thawing. This kit can also be used in high-throughput assays in which all the PCR reagents need to be ready and stable for PCR setup at any time.

II. KIT CONTENTS

Kit Contents	Quantity	Components/Concentration
Green *Taq* DNA polymerase	100 µl	5 units/µl with stabilizers
10X Reaction Buffer (with Mg^{2+})	1.5 ml	500 mM KCl, 100 mM Tris HCl (pH 9.0 at 25°C), 15 mM $MgCl_2$, 1% Triton X-100 and stabilizers.
Stabilized dNTP	300 µl	10 mM with stabilizers
Protocol	1	

Green *Taq* DNA Polymerase and Stabilized dNTP are also available separately.
Green *Taq* DNA Polymerase, 100 µl (500 U), Cat. No. E00043
10X *Taq* Buffer (with Mg^{2+}), 1.5 ml, Cat. No. B0005
Stabilized dNTP Mix, 300 µl (10 mM each), Cat. No. C01689

III. KEY FEATURES

- **High Stability:** The kit remains stable for more than six months when stored at 4°C or for one month when stored at room temperature (25°C) without significant activity loss.
- **High PCR yield:** The green *Taq* DNA polymerase has longer enzyme half-life and therefore increases the PCR yield when amplifying long DNA.
- **Terminal Transferase Activity:** *Taq* DNA polymerase has terminal transferase activity that results in the addition of a single nucleotide (adenosine) at 3'-end of the extension product.

IV. STORAGE

This product is shipped at ambient temperatures. The kit will remain stable for at least six months if stored at 4°C and for at least one year if stored at -20°C.

V. EXAMPLES

1. The Green *Taq* DNA polymerase increases PCR yield when amplifying long DNA.

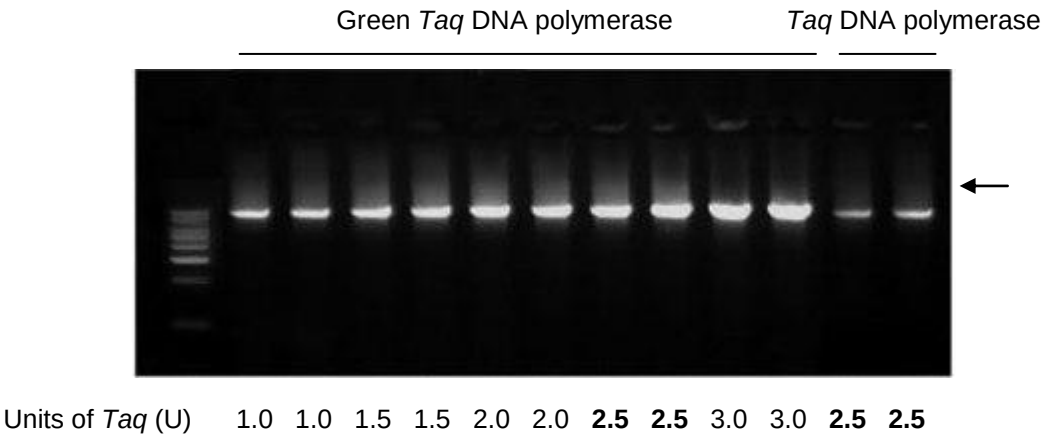

Figure 1. A 8-kb fragment from λDNA was amplified by using both *Taq* DNA polymerase and green *Taq* DNA polymerase. For long DNA fragment amplification, where longer elongation time is needed (in this case, the elongation time was three minutes), the green *Taq* DNA polymerase has a longer enzyme half-life and therefore increases the PCR DNA yield significantly.

2. The green *Taq* DNA polymerase can be stored at 37°C for 14 days without loss of activity.

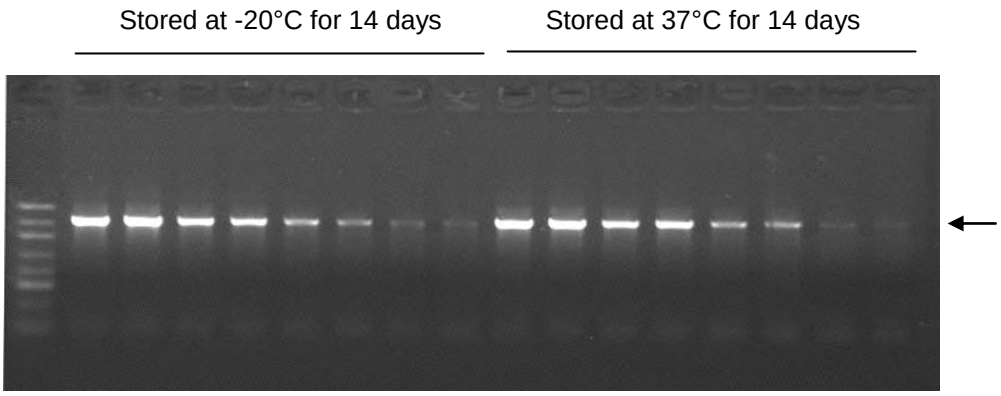

Figure 2. A 2-kb 23S rRNA fragment from from *E. coli* genomic DNA was amplified using green *Taq* DNA polymerase stored at -20°C and 37°C, respectively. The green *Taq* DNA polymerase can be stored at 37°C for 14 days without significant activity loss.

VI. GENERAL PCR PROTOCOL

1. Set up 50 µl PCR reaction in a thin-walled PCR tube on ice:

10X Reaction Buffer	5.0 µl
10 mM Stabilized dNTP	1.0 µl
Forward primer (20 µM)	1.0 µl
Reverse primer (20 µM)	1.0 µl
Template (up to 100 ng/µl)	2.0 µl
Sterile or filtered H_2O	39.5 µl
Green *Taq* DNA Polymerase	0.5 µl

2. Program PCR cycler as follows:

Initial denaturing:	94°C for three minutes
Then 30 cycles of:	94°C for 30 seconds
	55°C for 30 seconds
	72°C for 30 seconds to a few minutes (about 1 kb/minute)
Extension:	72°C for seven minutes

3. When the temperature of the PCR cycler reaches 94°C, place the PCR reaction in the PCR heating block and continue the program.
4. Analyze PCR fragments on an agarose or polyacrylamide gel.

Note:

1. This is a basic protocol. The reagent concentrations, conditions, and parameters may need to be optimized.
2. This protocol is for PCR cycler with a hot lid. Otherwise, mineral oil needs to be added to prevent evaporation.
3. 5% DMSO, 1M betaine, or both can be included in the PCR reaction to improve the results when a GC-rich template is used.

VII. ORDERING INFORMATION

High-Stability PCR Kit, Cat. No. L00342.

Green *Taq* DNA Polymerase, 100 µl (500 U), Cat. No. E00043

10X *Taq* Buffer (with Mg^{2+}), 1.5 ml, Cat. No. B0005

Stabilized dNTP Mix, 300 µl (10 mM each), Cat. No. C01689

For Research Use Only.

GenScript Corporation
120 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: info@genscript.com
Web: http://www.genscript.com