Apex™ Reagents and Chemicals
A Genesee Scientific Brand

*******SAMPLE PACK*******

Hot Start DNA Polymerase

Cat #: SMP42-107

Unit size: 50 Units

Buffers:
Buffer I – 10X Ammonium Reaction Buffer
750mM Tris-HCl, pH 8.5; $(NH_4)_2SO_4$
1% Tween® 20.
Buffer II – 10X Combination Reaction Buffer
Tris-HCl, pH 8.7; Balanced KCl/$(NH_4)_2SO_4$
1% Tween® 20.
50 mM $MgCl_2$ solution provided

Content: 1 x 50 units

Concentration: 5 units/µl

Storage: -20°C.
Reagent for *in vitro* laboratory use only

General Description

Apex Hot Start DNA Polymerase is a modified form of **Apex** Taq DNA Polymerase, which is activated by heat treatment. A chemical moiety is bound to the enzyme at the active site, which renders the enzyme inactive at room temperature. Thus, during setup and the first ramp of thermal cycling, the enzyme is not active and misprimed primers are not extended. Once the reaction reaches optimal activating temperature during a 15-minute heat activation step, the chemical moiety is cleaved, and the active **Apex** Hot Start DNA Polymerase is released into the reaction. The result is higher specificity and greater yields when compared to standard DNA polymerases.

Higher sensitivity improves multiplex PCR, an applied PCR technique that amplifies several specific targets simultaneously. Applications that previously required two or more reactions can be performed in a single reaction tube. Hence, multiplexing represents a substantial savings of time, supplies and costly reagents.

Apex Hot Start Storage Buffer

Enzyme is supplied in 20 mM Tris-HCl pH 8.3, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween® 20, 50% glycerol.

Key Features

- **Apex** Hot Start enzyme for increased specificity and product yield
- Successful multiplex reactions save time and reagents
- Designed to diminish the formation of non-specific product
- Detection of low target copy number

Unit Definition

One unit is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-precipitable form in 30 minutes at 72°C under standard assay conditions.

Quality Control

Endonuclease and exonuclease activities are not detected after 3 hours' incubation of 1 µg of pUC19 plasmid DNA and 0.5 µg *Eco*R I digested lambda phage DNA at 72°C in the presence of 40 units of **Apex** Hot Start DNA Polymerase.

Protocol

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be individually determined.

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis.

1. Thaw 10X Apex Buffer I and/or 10X Apex Buffer II, dNTP mix, 50 mM $MgCl_2$ and primer solutions. **It is important to mix the solutions completely before use to avoid localized concentrations of salts**.

2. Prepare a master mix according to Table 1. The master mix typically contains all the components needed for extension except the template DNA.

Table 1. Reaction components (Master Mix and Template DNA) for a 50µl reaction

Component	Vol./reaction	Final Conc.
10X Apex Buffer I or 10X Apex Buffer II	5 µl	1X
dNTP mix (100 mM of each)	0.5 – 1 µl	- - - -
$MgCl_2$ (50 mM)	0.5-5 µl	0.5-5mM
Primer A	Variable	0.1–1.0 µM
Primer B	Variable	0.1–1.0 µM
Apex Hot Start DNA Polymerase	0.5 - 1 µl	2.5 - 5 units
PCR Grade Water	Variable	- - - -
Template DNA	Variable	Variable
TOTAL volume	50 µl	- - - -

Table 2. $MgCl_2$ concentration in a 50 µL reaction

Final $MgCl_2$ conc. in reaction (mM)	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Additional volume of 50 mM $MgCl_2$	0.5	1.0	1.5	2.0	2.5	3.0	3.5

- In some applications, more than 1.5 mM $MgCl_2$ is needed for best results. Table 2 provides the volume of 50 mM $MgCl_2$ to add to the master mix if a higher $MgCl_2$ concentration is required.

3. Mix the master mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, *e.g.*, by pipetting the master mix up and down a few times.

4. Add template DNA to the individual tubes containing the master mix.

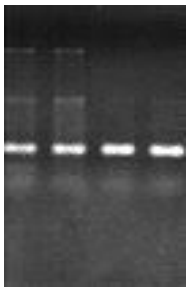
5. Program the thermal cycler according to the manufacturer's instructions. **Each program must start with an initial heat activation step at 95°C for 15 minutes.**

   For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

6. Place the tubes in the thermal cycler and start the reaction.

Figure 1. Comparison of Taq DNA polymerase with Apex Hot Start DNA Polymerase.

Under standard amplification conditions, a 355 bp DNA fragment was amplified using either a standard Taq DNA polymerase or Apex Hot Start DNA Polymerase.



1 2 3 4

Lane 1 5 units of Taq DNA Polymerase
Lane 2 2.5 units of Taq DNA Polymerase
Lane 3 5 units of **Apex** Hot Start Polymerase, with 15 minutes activation step at 95°C before cycling
Lane 4 2.5 units of **Apex** Hot Start Polymerase, with 15 minutes activation step at 95°C before cycling

Related Products

Taq Polymerase kits (500 units)	Cat#
With 10X Standard and Ammonium Reaction Buffer	42-800B1
With 10X Combination Buffer	42-800B3
Glycerol Free	42-800B4

Hot Start DNA Polymerase (500 units)	Cat#
With 10X Ammonium and Combination Reaction Buffer	42-106

High Fidelity - Proof reading (500 units)	Cat#
Hi-Fi PR™ Taq 2.5 U/µl	42-110

All polymerases are also available in kits, Mg^{2+} free buffers and 50 mM $MgCl_2$.

Master Mixes (500 reactions)	Cat#
2X Taq DNA Polymerase Master Mix, 1.5 mM $MgCl_2$	42-132
2X Taq RED Master Mix, 1.5 mM $MgCl_2$	42-138
2X Hot Start Master Mix Buffer I, 1.5 mM $MgCl_2$	42-198

The shown master mixes are ammonium based. Also available with balanced ammonium and potassium based buffers.

Real-time PCR (400 reactions)	Cat#
qPCR 2X Master Mix for Probe, without ROX™	42-116P
qPCR 2X Master Mix for Probe, low ROX™	42-118P
qPCR 2X Master Mix for Probe, high ROX™	42-120P
qPCR 2X GREEN Master Mix, without ROX™	42-116PG
qPCR 2X GREEN Master Mix, low ROX™	42-118PG
qPCR 2X GREEN Master Mix, high ROX™	42-120PG

Ultrapure dNTPs	Cat#
dNTP set, 100 mM each: 250 µl of each dA, dC, dG and dT	42-410
dNTP Set, 100 mM each: 1 ml of each dA, dC, dG and dT	42-403
dNTP Mix 40 mM (1 x 500 µl): 10 mM each dA, dC, dG, dT	42-411
dNTP Mix 100 mM (2 x 1 ml): 25 mM each dA, dC, dG, dT	42-405
dNTP Mix 10 mM (10 x 1 ml): 2.5 mM each dA, dC, dG, dT	42-406

Other concentrations and Single dNTPs are available.

DNA Ladders	Cat#
Apex 100 bp-Low DNA Ladder, 250 applications	19-109
Apex 1 kb DNA Ladder, 333 applications	19-115
Apex 200 bp DNA Ladder, 200 applications	19-111
Apex ECON Mini DNA Ladder, 100 applications	19-130
Apex ECON Low DNA Ladder, 100 applications	19-131
Apex ECON PCR Ladder, 100 applications	19-132

Accessory reagents	Cat#
50 mM $MgCl_2$, 3 × 1.5 ml	42-303
Nuclease-Free Water, PCR Grade, 6 x 5 ml	42-710