RAS Real *Taq* DNA Polymerase

(Modified DNA Polymerase)

5,000 U/ml	1600 units	Lot no xxxxxxxxx	Exp bcbcbbc	Store at -20C

Description

RAS Real Taq DNA Polymerase is a modified form of thermostable DNA polymerase from the thermophilic bacterium *Thermus aquaticus*. The enzyme catalyzes 5'→3' synthesis of DNA, has no detectable 3'→5' exonuclease (proofreading) activity and possesses 5'→3'exonuclease activity. It possesses high specificity and subsequently increases the yield of the specific PCR product. This is due to the reduced mis-primed products and primer dimers at ambient temperature as it is provided in an inactive state, thus it requires incubation at 95 °C for 15 minutes for activation.

Source

Produced from an *E. coli* strain that possesses the *Taq* DNA Polymerase gene from *Thermus aquaticus* that subsequently modified chemically.

Applications

- Routine PCR amplification of DNA fragments
- Quantitative PCR
- Nested PCR
- RFLP

Supplied in: 20mM HEPES, 100Mm KCl , 0.1 mM EDTA,0.5mM PMSF , 1 mM DTT, 50% Glycerol

Concentration

5U/µL

Reagents Supplied with Enzyme

- 50 mM $MgCl_2$
- 10x Hot Start Buffer
  200 mM Tris HCl (pH 8.8),
  500 mM KCl,
  160 mM $(NH_4)_2S0_4$
  Other additives

Storage conditions

- The recommended storage condition is -20°C.
- Avoid keeping at room temperature for long and repeated freeze thaws.

Quality Control

Quality Control assays

Mis-priming assay

No detectable non-specific bands were observed in agarose gel after PCR carried out to amplify E.coli gene from plasmid in Real taq buffer using 2.5 units of Real *Taq* DNA polymerase and gene specific primers in presence of back ground human genomic DNA (250 ng/µl).

Amplification efficiency

Amplification efficiency (AE) of RAS Real *Taq* tested on real-time PCR using quantitative standards belonging to various viruses yielded a slope between -3.1 and -3.6 (AE= 0.9-1.1).

DNase activity

No detectable change in banding pattern was observed in agarose gel after incubating the 25 units of Real *Taq* DNA Polymerase with 500 ng of phage DNA in RAS *Taq* reaction buffer at 37°C for 4 hrs.

RNase activity

Incubation of Human RNA (250 ng) with 25 units of Real *Taq* DNA polymerase in RAS *Taq* reaction buffer at 37°C for 1 hr results in no detectable change in banding pattern as determined by agarose gel electrophoresis.

E. *coli* DNA contamination

Taq polymerase is tested free of *E.coli* DNA contamination

Assay Conditions
Recommended PCR Reaction Mix (Real-Time)

Component	Volume (µL)
10x Hot Start reaction Buffer	2.5
$MgCl_2$ (50 mM)	1.0
Primer-Probe Mix	2.0
Template DNA	variable
Hot Start *Taq* DNA Polymerase	0.5
Molecular Grade Water	Make up to 25

Recommended Thermocycling conditions

Operation	Temp	Time	Cycles
Initial denaturation **	95˚C	15 min	1
Denaturation	95˚C	15-30 s	35-45 cycles
Annealing& extension	Tm-5˚C	60 s	

** Initial denaturation for 15 min at 95˚C is necessary for activation of enzyme.

Recommended PCR Reaction Mix (End point)

RAS Real *Taq* DNA Polymerase

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Component	Volume (µL)
10x Hot Start reaction Buffer	2.5
$MgCl_2$ (50 mM)	1.0
Forward primer (10 pmol/µL)	0.4-0.7
Reverse primer (10 pmol/µL)	0.4-0.7
Template DNA	variable
Hot Start *Taq* DNA Polymerase	0.5
Molecular Grade Water	Make up to 25

Recommended Thermocycling conditions

Operation	Temp	Time	Cycles
Initial denaturation **	95˚C	15 min	1
Denaturation	95˚C	30 s	30-35 cycles
Annealing	Tm-˚C	30-60 s	
Extension	72˚C	1 min/kb	
Final Extension	72˚C	5-10 min	--

** Initial denaturation for 15 min at 95˚C is necessary for activation of enzyme.

References

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2. Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) *Biokhimiya*, 45, 644-651.
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4. Longley, M.J., Bennett, S.E. and Mosbaugh D.W. (1990) *Nucleic Acids Res.*, 18, 7317-7322.