RAS Lifesciences Pvt Ltd
Striving for better health

RAS *Taq* Enzy

(DNA Polymerase)

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

Description

RAS *Taq* DNA Polymerase is a thermostable DNA polymerase that possesses 5'→3' polymerase and exonuclease activity, but lacks 3'→5' exonuclease (proofreading) activity.

Source

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

Applications

- PCR
- TA cloning
- RFLP
- High-throughput PCR
- Primer extension
- Microarray analysis

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

Concentration

5 U/µL

Unit definition

One unit is the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTP into acid-insoluble material in 30 minutes at 65°C.

Reagents Supplied with Enzyme

- **10X RAS *Taq* Reaction Buffer**

  670 mM Tris HCl (pH 8.8)

  160 mM $(NH_4)_2SO_4$,

  25mM $MgCl_2$,

  0.1% Tween 20

Quality Control assays

PCR for 5kb amplicon

PCR amplification using 2.5 U of RAS *Taq* polymerase with 5 ng of plasmid DNA in presence of 200 uM dNTPS and 10 picomoles primers in *Taq* reaction buffer results in the expected 5kb product.

DNase activity

No detectable change in banding pattern was observed in agarose gel after incubating the 25 units of *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 *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

Storage conditions

- The recommended storage condition is -20°C.

References

1. Chien, A., Edgar, D.B. and Trela, J.M. (1976) *J. Bact.*, 127, 1550-1557.
2. Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) *Biokhimiya*, 45, 644-651.
3. Lawyer, F.C. et al. (1993) *PCR Methods and Appl.*, 2, 275-287.
4. Longley, M.J., Bennett, S.E. and Mosbaugh D.W. (1990) *Nucleic Acids Res.*, 18, 7317-7322.

Presentation

Cat. No	Units	Enzyme	Buffer
ENTQE-100	100 Units	1 x 20 µL	1 x 100 µL
ENTQE-500	500 Units	1 x 100 µL	1 x 500 µL
ENTQE-1k	1000 Units	1 x 200 µL	1 x 1.0 mL
ENTQE-5k	5000 Units	1 x 1.0 mL	1 x 5.0 mL

For further information on protocols and details for RAS *Taq* DNA Polymerase reaction, please contact our technical support: info@raslifesciences.com

PCR guidelines: Polymerase Chain Reaction is an efficient and sensitive technique for apmlification of DNA. *Taq* DNA polymease is

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routinely used in PCR. Few guidelines are presented below to achive a successful PCR employing RAS *Taq* enzyme. However, further optimization may require for amplification of amplicons greter than 5 kb and templates with high GC content and low template concentrations.

PCR set-up: prepare PCR reactions on ice using required volume of freeze-thawed components in the PCR hood as mentioned in the table below

Component	Volume (µL)
10X *Taq* Reaction Buffer	2.5
10 mM dNTPs	0.5
Forward primer (10 pmol/µL)	0.4-0.7
Reverse primer (10 pmol/µL)	0.4-0.7
Template DNA	X µL
RAS *Taq* DNA Polymerase	0.5
Molecular Grade Water	Make up to 25

Note: gently mix the reaction mixture and perform a short-spin to collect all liquid to bottom.

Immediately transfer the reaction tubes from ice to pre-heated thermocycler and start thermocycling.

Recommended PCR conditions

Operation	Temp	Time	Cycles
Initial denaturation	95˚C	1-5 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	--

Tips for successful PCR

Avoiding cross-contamination: bulk product is generated from a single copy in PCR. Thsu, care must be taken to avoid cross-contamination among the amplicons that spread in the lab environment. Few precautions are recommended to minimize the risk of contamination which are follows as:

- Prepare PCR reactions in a laminar flow hood equipped with UV lamp.
- PCR mix preperation, sample addition, thermocycling and gel-electrophoresis should be conducted in separate areas.
- Use PCR grade reagents
- Wear gloves while setting up the reaction.
- Use filtered tips to avoid aerosol contamination.

Reaction components & cycling conditions

Mg ions: the concentration between 1.5 and 2.5 mM is recommended for routine PCR. RAS real taq effectively amplify the target with 1.5 mM mgcl2. However, it needs to be optimized for DNA samples containing chelating agents that may require more $MgCl_2$. Very high levels mg promote non specificity while low concentration reduce the yield.

Additive: The PCR affected by presence of the inhibitors in the samples. That can overcome using the PCR facilitators like DMSO, betaine etc.

Template

Optimal DNA template concentration usually used in PCR up to 1 ng for both plasmid and phage DNA while 1 g for genomic DNA. The higher concentrations of template DNA generate non-specific PCR products and lower concentrations affect the PCR accuracy.

dNTPs

Generally, 200 um of each dNTP is recommended in PCR. However, in few occasions may require more dNTP concentration where Mg concentration needs to be adjusted accordingly as Mg binds to dNTPs.

Primers

The suggested concentration of primers in PCR ranges between 0.1 and1 µM. The higher concentrations result in generation of non-specific PCR products.

Initial denaturation

The initial denaturation helps in unwinding the template completely that facilitates effective amplification during the first cycles which subsequently carried over in further cycles. The recommended initial denaturation time is 1-3 min at 95˚C when GC content is below 50%. This step can be prolonged to 10 min for GC rich templates.

Denaturation

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30 seconds of denaturation time per cycle is sufficient, however, it can be prolonged for GC rich templates (1-3 min).

Primer annealing

This step is typically 30-60 seconds. The annealing temperature should be 5˚C less than the primers melting temperature (Tm). Melting temperature should be optimized by increasing the temperature gradually if un-desired products appear.

Extension

The optimal temperature for enzyme activity is observed between 70 and 75°C. We recommend 72°C for extension of the template. Extension times are generally 1 minute per kb. A final extension of 5-10 minutes at 72°C is sufficient to fill-in any possible incomplete reaction products..

Cycle number

Generally 25-35 cycles are recommended to obtain the product sufficiently. Up to 45 cycles may be required to detect low-copy-number targets.

PCR product

The PCR product obtained using RAS *Taq* DNA polymerase contains dA overhangs at the 3´–end; therefore the PCR products can be used in TA cloning.

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