RAS Real *Taq* Mix

(Modified DNA Polymerase)

Lot no xxxxxxxxx **Exp bcbcbbc** **Store at -20C**

Description

2X RAS Real *Taq* Mix is a ready to-use optimized solution preferred for routine real-time PCR. However, it also can be used in conventional PCR. It contains hot-start *Taq* DNA Polymerase (RAS Real *Taq* enzyme), dNTPs, $MgCL_2$ and reaction buffer at optimal concentrations. The presence of hot-start *Taq* DNA Polymerase significantly improves the specificity of the mix that 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. RAS Real *Taq* mix works effectively even in presence of inhibitors.

Applications

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

Concentration

2X

Composition of the Mix

- RAS Real *Taq* (hot-start) enzyme (200 U/ml)
- Reaction buffer
- 0.4 mM dNTP mix
- BSA

Storage conditions

- The recommended storage condition is -20°C.
- Store the mix in working aliquots to minimize the repeated freeze thaw cycles.
- Mix well prior to use.

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).

Nuclease assays: No contaminating nucleases in the mix were detected.

E. *coli* DNA contamination: No contaminating E. *coli* DNA was detected in the mix.

Assay Conditions
Recommended PCR Reaction Mix (Real-Time)

Component	Volume (µL)
2x RAS Real *Taq* Mix	12.5
Primer-Probe Mix	2.0
Internal control	1.0
Template DNA	variable
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)

Component	Volume (µL)
2x RAS Real *Taq* Mix	12.5
Forward primer (10 pmol/µL)	0.4-0.7
Reverse primer (10 pmol/µL)	0.4-0.7
Template DNA	variable
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.

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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.