RAS Lifesciences Pvt Ltd
Striving for better health

RAS PCR MIX (5X)

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

Description

RAS PCR Mix (5x) is a ready to-use optimized solution for routine PCR. It contains high quality recombinant *Taq* DNA Polymerase, dNTPs, $MgCL_2$ and reaction buffer at optimal concentrations enabling effective amplification of DNA targets by PCR. RAS PCR mix works effectively even in presence of inhibitors.

Applications

- Routine PCR Assays
- Amplifying cDNA
- Generating PCR products for TA cloning

Concentration

5X

Composition of the mix

- RAS *Taq* DNA polymerase (250 U/ml)
- Reaction Buffer (pH)
- 7.5 mM $MgCl_2$
- 1.0 mM dNTP mix
- Other additives

Quality Control

Amplification test

PCR amplification with RAS PCR mix in the presence of primers results in the expected 2kb product.

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

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.

References

1. Dieffenbach CW, Dveksler GS (eds) PCR Primer: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
2. Erlich HA (ed) (1989) PCR Technology: Principles and Applications for DNA Amplification. Stockton Press, New York
3. Innis MA, Gelfand DH, Sninsdy JJ (eds) (1995) PCR Strategies. Academic Press, San Diego, California
4. Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) (1990) PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, California
5. Newton CR, Graham A (1994) PCR. BIOS Scientific Publishers, Oxford
6. Watson JD, Gilman M, Witkowski J, Zoller M (1992) Recombinant DNA, second edition.

Presentation

Cat. No	Rxns	Enzyme
ENPCX-100	100 rxns	1 x 0.50mL
ENPCX-250	250 rxns	1 x 1.25mL
ENPCX-500	500 rxns	2 x 1.25mL
ENPCX-5K	5000 rxns	20 x 1.25mL

PCR guidelines: Polymerase Chain Reaction is an efficient and sensitive technique for apmlification of DNA. *Taq* DNA polymease is routinely used in PCR. Few guidelines are presented below to achive a successful PCR employing RAS *Taq* PCR mix. However, further optimization may require for amplification of amplicons greater than 2 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)
RAS PCR Mix (5x)	5
Forward primer (10 pmol/µL)	0.4-0.7
Reverse primer (10 pmol/µL)	0.4-0.7
Template DNA	X µL
MBGW	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-5˚C	30-60 s	
Extension	72˚C	1 min/kb	
Final Extension	72˚C	5-10 min	--

Tips for successful PCR

Manufactured by:

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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.

Template

Optimal DNA template concentration usually used in PCR up to 1 ng for both plasmid and phage DNA while 1ug 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 which is incorporated in the RAS PCR mix. 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 and 1 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

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.

For further information on protocols and details for RAS PCR Mix reaction, please contact our technical support: info@raslifesciences.com

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