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2X PCR Master Mix

400 REACTIONS (25µl each)

Store at -20°C

PN # MBK0013

Product description

2X PCR Master Mix has been optimized for high throughput PCR and use in routinely application such as detection of DNA, bacterial colonies and cDNA products. This ready-to-use solution is fast-saves time and minimizes contamination, due to reduced number of pipetting steps. The mix is optimized for efficient and reproducible PCR and qualitative as quantitative results are guaranteed. The mix can amplify templates in the range of 0.1-2 Kb.
PCR products generated with 2X PCR Master Mix contain mostly 3'-A overhangs ends and so they can be directly cloned into T-vector.
2X PCR Master Mix may be used for difficult templates such as GC-rich DNA sequences and also provides robust performance in PCR multiplex.
The provided enzyme is designed for hot start PCR allowing a robust and specific amplification and a easier assembly of the reaction mixtures at room temperature.

Kit contents

•**2X PCR Master Mix** for PCR analyses with optimized buffer components (dNTP mixture and magnesium chloride included).
•**Hot-Rescue DNA Polymerase** prevents the primer-dimer forms and non specific products, increasing the target yield.
•**$MgCl_2$ (25 mM)** for easy optimization of PCR performance**.** It is well known that the magnesium concentration affects primer annealing, product specificity and enzyme activity. Generally, $MgCl_2$ final concentration of 2mM is sufficient for most reactions, but an optimal Mg++ concentration (reaching to 5-6 mM) increases sensitivity and performance's reaction.

4 x 1250 µl 2X PCR Master Mix
1 x 250 U Hot-Rescue DNA Polymerase (5U/ul)
1 x 1 mL $MgCl_2$ (25 mM)

Storage

–20°C

General protocol for PCR set up

Set up the experimental reaction by adding the following components:

For 1 REACTION		
	Volume/reaction	*Final concentration*
Nuclease-free PCR-grade H_2O	Variable	-
2X PCR Master Mix *	12,5µl	1X
25mM $MgCl_2$ solution	Variable	Up to 6 mM
Upstream primer	Variable	0.1-0.4 µM
Downstream primer	Variable	0.1-0.4 µM
DNA template	Variable	Preferably **10^9-100** target copies; or **10-300** ng of DNA or **10-40** ng of cDNA
Hot-Rescue DNA Polymerase	0,125µl	0,625 Units/reaction
Total volume	**25 µl**	
Cycle Number:	**30 – 50**	Cycle number depends on the amount of template DNA

* Provides a final concentration of 2mM $MgCl_2$.

For different volumes, calculate all components proportionally.

PCR Cycle program

Run the appropriate PCR program as indicated below:

STEP	TIME	TEMP	*Additional Comments*
Initial activation step	1 min	95°C	DNA polymerase activity is released during this short heating step
Denaturation	15 s	95°C	-
Annealing*	10-30 s	x°C	Approximately 5 to 8°C below *T*m of primers
Extension*	15-45 s	60-72°C	
Cycle number	30-50		Number of cycles depends on the amount of template DNA

*Set up a single step at ≥ 60° C for 30-60 second when the Tm of primers is similar to the Tm extension temperature.

Agarose gel Electrophoresis

Load and separate PCR products on an agarose gel at 5 – 8 V/cm. DNA bands can be stained and visualized with standard staining methods.

Troubleshooting

Observation	Possible Cause	Suggested Solution
No PCR product	Missing components (e.g. primers, template or DNA Polymerase).	Check the assembly of the reaction.
	Missing essential step in the Cycler protocol.	Check the cycler protocol.
	Insufficient starting template.	Increase template amount if possible.
	Primer concentration too low.	Increase primer concentration.
	Primer degraded.	Check for primer degradation.
	Insufficient extension time for the amplicon size.	Increase the extension time.
	Poor quality template.	Organic extraction followed by ethanol precipitation may remove some inhibitors of amplification.
Multiple PCR products	Primer concentration too high.	Reduce primer concentration.
	Magnesium chloride concentration not optimal.	Vary magnesium chloride concentration up to 6mM