

2x PCR Master mix Series

2x PCR Master mix Solution (i-MAX II)

Cat. No. 25266 (1ml)

DESCRIPTION

iNTRON's *Maxime* PCR PreMix Kit has not only various kinds of PreMix Kit according to experience purpose, but also a 2X Master mix solution.

2x PCR Master mix Solution (i-MAX II) is made from iNTRON's i-MAX™ II DNA Polymerase.

Taq DNA Polymerase is the most common PCR enzyme for amplifying up to 10kb λ DNA templates and up to 3kb genomic templates. However, it is not able to amplify even longer DNA fragments. To overcome this constraint, variable PCR systems have been developed by various company, which are containing *Taq* DNA Polymerase and thermostable DNA Polymerase with proofreading activity. i-MAX™ II DNA Polymerase is the PCR System which are developed for amplifying long and complex fragments. The first is designed for amplification of 5-10kb fragments from genomic DNA. The second (i-MAX™ II DNA Polymerase) can amplify even longer fragments up to 20kb from human genomic DNA, and up to 30kb from a λ DNA template). Moreover, the second have improved amplification efficiency compared to i-MAX™ DNA Polymerase by improving enzyme activity. Therefore i-MAX™ II DNA Polymerase is a more versatile enzyme blend than i-MAX™ DNA Polymerase in amplifying various template including short and long DNA fragment or simple and complex DNA, either.

2x PCR Master mix Solution (i-MAX II) is the product what is mixed every component : i-MAX II DNA Polymerase, dNTP mixture, reaction buffer, and so on- in one tube. This is the product that can get the best result with the most convenience system. The first reason is that it has every components for PCR, so we can do PCR just add a template DNA, primer set, and D.W.. The second reason is that it has Gel loading buffer to do electrophoresis, so we can do gel loading without any treatment. In addition, each batches are checked by a thorough Q.C., so its reappearance is high. It is suitable for various sample's experience by fast and simple using method.

STORAGE

Store at 4°C; under this condition, it is stable for at least a year.

CHARACTERISTICS

- Increased fidelity of PCR amplification : because the i-MAX™ II DNA Polymerase enzyme blend combines the proofreading activity of Pfu DNA Polymerase with the high processivity of *Taq* DNA Polymerase.
- Increased yield of PCR amplification : because the accuracy of the enzyme blend reduces the number of truncated amplification products formed.
- Improved performance of long PCR : because the reaction buffer and the enzyme blend are optimized for generation of certain length products
- Flexibility : available for various DNA template including cloned fragment, phage DNA, mammalian genomic DNA and etc.
- Ready to use: only template and primers are needed
- Construct as various reaction volume
- Stable for over 1 year at 4°C
- Time-saving and cost-effective

CONTENTS

• 2x PCR Master mix Solution (i-MAX II)	1 ml
i-MAX II DNA Polymerase(5U/ μ l)	
dNTPs	
Reaction Buffer	
Gel Loading buffer	

Note : The PCR process is covered by patents issued and applicable in certain countries. iNTRON Biotechnology does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

PROTOCOL

1. Dispense 10 μ l of 2x PCR Master mix Solution (in case of total 20 μ l PCR reaction) / 25 μ l of 2x PCR Master mix Solution (in case of total 50 μ l PCR reaction) into PCR tubes.
2. Add template DNA and gene specific primers into upper PCR tubes.
 - Note 1 : Appropriate amounts of DNA template samples
 - cDNA : 0.5-10% of first RT reaction volume
 - Plasmid DNA : 10pg-100ng
 - Genomic DNA : 0.1-1ug for single copy
 - Note 2 : Appropriate amounts of primers
 - Primer : 5-20pmol/ μ l each (sense and anti-sense)
3. Add distilled water into the tubes to a total volume of 20 μ l or 50 μ l.

Example	20 μ l Rxn	50 μ l Rxn
PCR reaction mixture	Add	Add
2x PCR Master mix Soln.	10 μ l	25 μ l
Template DNA	1 - 2 μ l	1 - 2 μ l
Primer (F : 10pmol/ μ l)	1 μ l	1 μ l
Primer (R : 10pmol/ μ l)	1 μ l	1 μ l
Distilled Water	6 - 7 μ l	21 - 22 μ l
Total reaction volume	20μl	50μl

Note : This example serves as a guideline for PCR amplification. Optimal reaction conditions such as amount of template DNA and amount of primer, may vary and must be individually determined.

4. Mix the mixture thoroughly.
5. (Option) Add mineral oil.
 - Note : This step is unnecessary when using a thermal cycler that employs a top heating method (general methods)
6. Perform PCR of samples.
7. Load samples on agarose gel without adding a loading-dye buffer and perform electrophoresis.

SUGGESTED CYCLING PARAMETERS

Cycle program for fragments < 10kb			
	Temp.	Time	Cycle No.
Initial Denaturation	92-94°C	2-4min	1
Denaturation	94°C	15s-1min	25-30
Annealing	45-65°C	15s-1min	
Extension*	72°C	1min / 1-1.5kb	
Final extension	72°C 4°C	5-10min hold	1
*, Extension time for 30s-1min is sufficient for fragments < 1kb.			
Cycle program for fragments > 10kb			
	Temp.	Time	Cycle No.
Initial Denaturation	92-94°C	2-4min	1
Denaturation	94°C	15s-1min	10
Annealing	45-65°C	15s-1min	
Extension	72°C	1min / 1-1.5kb	
Denaturation	94°C	15s-1min	15-20
Annealing	45-65°C	15s-1min	
Extension	72°C	1min / 1-1.5kb + 20s / cycle	
Final extension	72°C 4°C	5-10min hold	1

Note : This CYCLING PARAMETERS serves as a guideline for PCR amplification. optimal reaction conditions such as PCR cycles, annealing temperature, extension temperature and incubation times, may vary and must be individually determined.

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