DATA SHEET

2x PCR Master mix Series

2x PCR Master mix Solution (i-pfu)

Cat. No. 25186 (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. Pfu DNA Polymerase exhibits the lowest error rate of any thermostable DNA polymerase studied. For routine PCR, where simple detection of an amplification product or estimation of the product's size is important, Taq DNA polymerase is the obvious enzyme to choose. However, when the amplified product is to be cloned, expressed or used in mutagenesis studies, Pfu DNA polymerase is a much better enzyme of choice for PCR. 2x PCR Master mix Solution (i-pfu) is made from iNtRON's i-Pfu DNA Polymerase. i-Pfu DNA Polymerase is a thermostable DNA polymerase purified from an E.coli strain carrying a plasmid with the cloned gene encoding Pyrococcus furiosus DNA polymerase.

The enzyme catalyzes the incorporation of nucleotides into duplex DNA in the 5'=>3' direction in the presence of Mg 2+ at 70-80¶C. Pfu DNA Polymerase exhibits 3'=>5' exonuclease (proofreading) activity, but has no detectable 5'=>3' exonuclease activity. 2x PCR Master mix Solution (i-pfu) is the product what is mixed every component : i-pfu 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

• High Fidelity : presence of 3'ç5' exonuclease (proofreading)

• Low Error : the lowest error rate of any thermostable DNA polymerase studied.

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• 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×

• Time-saving and cost-effective

CONTENTS

• 2x PCR Master mix Solution (*i*-pfu)	1 ml
i-Pfu DNA Polymerase(2.5U/μl) 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 10μl of 2x PCR Master mix Solution (in case of total 20μl PCR reaction) / 25μl of 2x PCR Master mix Solution (in case of total 50μl 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/μl each (sense and anti-sense)

3. Add distilled water into the tubes to a total volume of 20μl or 50μl.

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

PCR cycle		Temp.	PCR product size	
			≤2kb	≥2kb
Initial denaturation		94 ℃	2min	2min
30-40 Cycles	Denaturation	94 ℃	20sec	20sec
	Annealing	50-65 ℃	10sec	10sec
	Extension	65-72 ℃	30sec ~ 1min/kb	1min 30sec ~ 2min/kb
Final extension		72 ℃	Optional. Normally, 2-5min	

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.

EXPERIMENTAL INFORMATION

• *Comparison with different company kit*

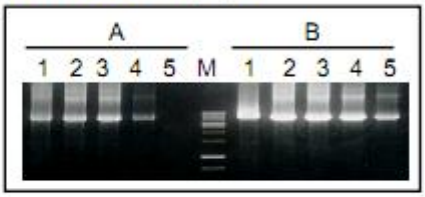

Fig.1. Comparison of 2x PCR Master mix Solution (*i*-pfu) and company A's 2x master mix solution system by amplifying 9Kb DNA fragment from variable amounts of SLT plasmid DNA .

A, Company A; **B,** 2x PCR Master mix Solution (*i*-pfu)
Lanes M, 1Kb Marker; **lanes 1,** 250 ng DNA; **lane 2,** 50 ng DNA; **lane 3,** 10 ng DNA; **lane 4,** 2 ng DNA; **lane 5,** 400 pg DNA