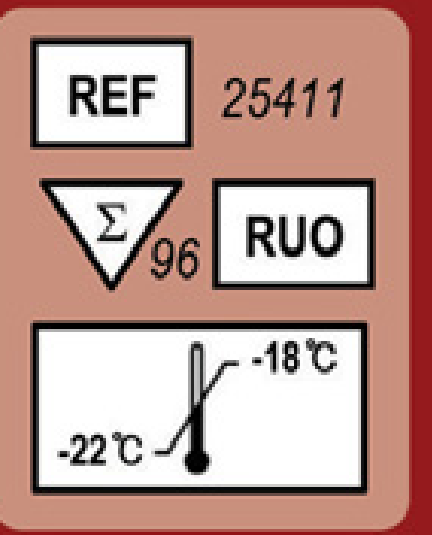
ISO 9001 | ISO 14001

Instruction Manual

FastMix™ FRENCHE

PCR (i-StarTaq)



The Best Choice of FastMix for PCR work

For highly specific hot-start PCR
without the need for optimization

iNtRON Biotechnology

www.intronbio.com
www.intron-innoplex.com

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DESCRIPTION

RTS Technology is iNtRON's innovative and original technology which is adapted to manufacture PCR Premix. It is a platform technology that guarantees the effective activity and stability of PCR premix even in the room temperature or extreme circumstance.

FastMix / Frenche™ PCR (i-StarTaq) based on this RTS Technology is designed to keep the stable performance of product up to six months at room temperature and it is adapting all-in-one type which has all components dried in the PCR tube for PCR amplification. This kit is very convenient to use with high specificity and sensitivity with hot-start PCR function and provides no different data depending on researchers. Also it is very safe from the cross contamination due to the vacuum packaging system.

FastMix / Frenche™ PCR (i-StarTaq), it is the another name of convenience in simplicity. The quality is guaranteed.

CHRACTERISTICS

- Simple and fast to handle
- Higher PCR specificity and reduced nonspecific amplification
- Fewer pipetting steps reduces the risk of contamination
- Ready to use
- Improved Stability

KIT CONTENTS

- **FastMix / Frenche™ PCR (i-StarTaq) Strip (8 Tubes)*** x 12 strips
- **Instruction Manual (Hand-book)** 1 ea

* Each of Strip is supplied under vacuum packaging to protect oxidation, hydration and penetration of contaminant.

Components of FastMix / Frenche™ PCR (i-StarTaq)	
• Reaction Buffer	1 X
• dNTP Solution	250 μM
• $MgCl_2$	2.0 mM
• DNA Polymerase (i-StarTaq)	2.5 Units

STORAGE

- Storage condition : Store the product at -22 ~ -18℃ after receiving.
- Expiration : **FastMix / Frenche™ PCR (i-StarTaq)** can be stored for up to 24 months without showing any reduction in performance and quality under appropriate storage condition. The expiration date was labeled on the product box.
- The stability of the product confirmed at room temperature and harsh conditions. The logistic process is performed at room temperature, but the quality of the product is functioning properly

IMPORTANT NOTES BEFORE STARTING

- FastMix / Frenche™ PCR (i-StarTaq) provides a final concentration of 2 mM $MgCl_2$ which will produce satisfactory results in most cases. However, if a higher Mg^{2+} concentration is required, prepare a stock solution containing 25 mM $MgCl_2$.
- Set up reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

ADDITIONAL REQUIRED EQUIPMENT

- Distilled water
- Pipets and pipet tips (aerosol resistant)
- Mineral oil (only if the thermal cycler does not have a heated lid)
- Primers
- Thermal cycler

APPLICATIONS

- Complex genomic DNA PCR
- Complex cDNA templates : RT-PCR (2nd round PCR of RT-PCR)
- Very low-copy targets
- Reactions with multiple primer pairs

QUALITY CONTROL

- In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of FastMix / Frenche™ PCR (i-StarTaq) is tested against predetermined specifications to ensure consistent product quality.

Contents	Quality Control
PCR Buffer, dNTP Mixture	Conductivity, pH, sterility, and performance in PCR are tested.
Distilled Water	Conductivity, pH, sterility, and performance in PCR are tested. Endonuclease, exonuclease, and RNase activities are tested.
FastMix/Frenche™ PCR (i-StarTaq)	PCR reproducibility assay: The PCR reproducibility assay reactions are performed in parallel using FastMix/Freche PCR (i-StarTaq).
Process Inspection	Appearance of PCR Strip (housing, vacuum packaging was tested. Accuracy of aliquot process was validated. VDO process was validated

TECHNICAL ASSISTANCE

At iNtRON we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If you have any questions or experience any difficulties regarding the FastMix / Frenche™ PCR (i-StarTaq) or iNtRON products in general, please do not hesitate to contact us. iNtRON customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at iNtRON. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques. For technical assistance and more information please call iNtRON Technical Service Department or local distributors.

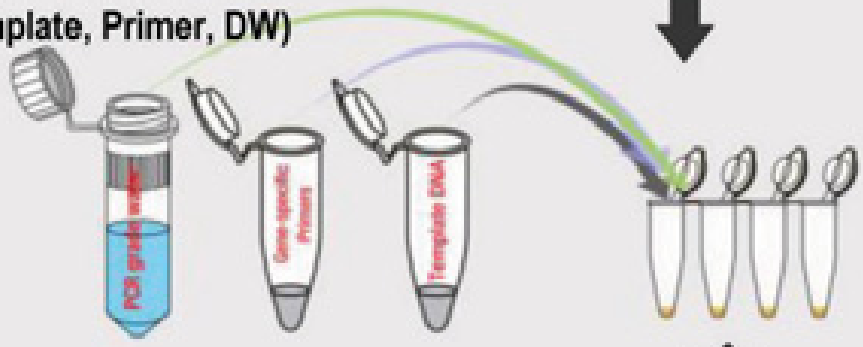
PRODUCT USE LIMITATIONS

FastMix / Frenche™ PCR (i-StarTaq) is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. FastMix / Frenche™ PCR (i-StarTaq) is developed, designed, and sold for research purpose only. They are not to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals.

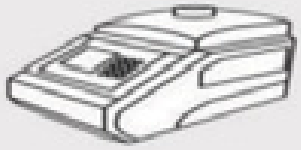
QUICK GUIDE

1. Prepare the Samples

2. Mix the Components
(Template, Primer, DW)



3. Run the PCR Cycle



4. Gel Loading

5. Analysis the Results

PROTOCOL

1. Add template DNA and primers into FastMix / Frenche™ PCR(i-StarTaq).

Note 1 : Recommended volume of template : 1 µl ~ 2 µl

Note 2 : Appropriate amounts of DNA template samples

- cDNA : 0.5-10% of first RT reaction volume
- Plasmid DNA : 10 pg-100 ng
- Genomic DNA : 0.1-1 µg for single copy

Note 3 : Appropriate amounts of primers

- Primer : 5-20 pmol/µl each (sense and anti-sense)

2. Add distilled water into the tubes to a total volume of 20 µl.

Example **Total 20 µl reaction volume**

PCR reaction mixture	Add
Template DNA	1 ~ 2 µl
Primer (F : 10pmol/µl)	1 µl
Primer (R : 10pmol/µl)	1 µl
Distilled Water	16 ~ 17 µl
Total reaction volume	**20 µl**

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.

3. **Dissolve the orange pellet by pipetting.**
   Note : If the mixture lets stand at RT for 1-2min after adding water, the pellet is easily dissolved.

4. **(Option) Add mineral oil.**
   Note : This step is unnecessary when using a thermal cycler that employs a top heating method(general methods).

5. **Perform PCR of samples.**
   Note : SUGGESTED CYCLING PARAMETERS

PCR cycle		Temp.	PCR product size		
			100-500 bp	500-1000 bp	1Kb-5 Kb
Initial denaturation		94 ℃	5~10 min	5~10 min	5~10 min
30-40 Cycles	Denaturation	94 ℃	20 sec	20 sec	20 sec
	Annealing	50-65 ℃	10 sec	10 sec	20 sec
	Extension	65-72 ℃	20-30 sec	40-50 sec	1 min/Kb
Final extension		72 ℃	Optional. Normally, 2-5 min		

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.

6. **Load samples on agarose gel without adding a loading-dye buffer and perform electrophoresis.**

TROUBLESHOOTING GUIDE

Symptoms & Possible Causes	Comments & Suggestions
Little or no product	
A. Pipetting error or missing reagent	Repeat the PCR. Check the concentrations and storage conditions of the kit, primers and template.
B. Primer concentration not optimal or primers degraded	Repeat the PCR with different primer concentrations from 0.1–0.5 µM of each primer (in 0.1 µM increments). In particular, when performing highly sensitive PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel.
C. Problems with starting template	Check the concentration, storage conditions, and quality of the starting template. If necessary, make new serial dilutions of template nucleic acid from stock solutions. Repeat the PCR using the new dilutions.
D. Insufficient number of cycles	Increase the number of cycles in increments of 5 cycles.

TROUBLESHOOTING GUIDE

Symptoms & Possible Causes	Comments & Suggestions
E. Pipetting error or missing reagent	Repeat the PCR. Check the concentrations and storage conditions of the kit, primers and template.
F. Incorrect annealing temperature or time	Decrease annealing temperature by 2°C increments. Annealing time should be between 30 and 60 s.
G. Incorrect denaturation temperature or time	Denaturation should be at 94°C for 30–60 s.
H. Extension time too short	Increase the extension time by increments of 1 min.
I. Insufficient starting template	Perform a second round of PCR using a nested-PCR approach
J. Primer design not optimal	Review primer design
K. RT reaction error	For RT-PCR, take into consideration the efficiency of reverse transcriptase reaction which averages 10–30%. The added volume of the reverse transcriptase reaction should not exceed 10% of the final PCR volume

TROUBLESHOOTING GUIDE

Symptoms & Possible Causes	Comments & Suggestions
L. PCR overlaid with mineral oil when using a thermal cycler with a heated lid	When performing PCR in a thermal cycler with a heated lid that is switched on, do not overlay the PCR samples with mineral oil, as this may decrease the yield of amplicon.
M. Problems with thermal cycler	Check the power to the thermal cycler and that the thermal cycler has been correctly programmed.
N. Too short Initial denaturation time	Increase the Initial denaturation time more than 5~10 mins
Product is multi-banded	
A. Annealing temperature too low	Increase annealing temperature in 2°C increments. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR
B. Primer concentration not optimal or primers degraded	Repeat the PCR with different primer concentrations from 0.1–0.5 µM of each primer (in 0.1 µM increments).
C. Primer design not optimal	Review primer design

TROUBLESHOOTING GUIDE

Symptoms & Possible Causes	Comments & Suggestions
Product is smeared	
A. Too much starting template	Check the concentration and storage conditions of the starting template. Make serial dilutions of template nucleic acid from stock solutions. Perform PCR using serial dilutions.
B. Carry-over contamination	If the negative-control PCR (without template DNA) shows a PCR product or a smear, exchange all reagents. Use disposable tips containing hydrophobic filters to minimize cross-contamination. Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
C. Too many cycles	Reduce the number of cycles in increments of 3 cycles.
D. Primer concentration not optimal or primers degraded	Repeat the PCR with different primer or primers degraded concentrations.

TECHNICAL ADVICE

◈ Number of PCR Cycles

A cycling program usually consists of 25–35 cycles, depending on the number of copies of the starting template. Increasing the number of cycles does not necessarily lead to a higher yield of PCR product; instead they may increase nonspecific background and decrease the yield of specific PCR product.

General Guidelines for Choosing the Number of Cycles

Number of copies of starting template	1 kb DNA	E. coli DNA	Human gDNA	Number of cycles
10 – 100	0.01–0.11 fg	0.05–0.56 pg	36–360 pg	40–45
100 – 1000	0.11–1.1 fg	0.56–5.56 pg	0.36–3.6 ng	35–40
$1 \times 10^3 - 5 \times 10^4$	1.1–55 fg	5.56–278 pg	3.6–179 ng	30–35
$> 5 \times 10^4$	>55 fg	>278 pg	>179 ng	25–35

◈ Nested PCR

If PCR sensitivity is low, nested PCR often improves product yield. This technique involves two rounds of amplification reactions. The first-round PCR is performed according to the PCR Protocol. Subsequently, an aliquot of the first-round PCR product, for example 1 µl of a 10^{-3} –10^{-4} dilution, is subjected to a second round of PCR. The second-round PCR is performed with two new primers that hybridize to sequences internal to the first-round primer–target sequences. In this way, only specific first-round PCR products will be amplified in the second round. Alternatively, it is possible to use one internal primer and one first-round primer in the second PCR

◈ Touchdown PCR

Touchdown PCR uses a cycling program with varying annealing temperatures. It is a useful method to increase the specificity of PCR. The annealing temperature in the initial PCR cycle should be 5–10°C above the estimated Tm of the primers. In subsequent cycles, the annealing temperature is decreased in increments of 1–2°C per cycle until a temperature is reached equal to, or 2–5°C below, the Tm of the primers. Touchdown PCR enhances the specificity of the initial primer–template duplex formation and hence the specificity of the final PCR product. To program your thermal cycler for touchdown PCR, you should refer to the manufacturer's instructions.

◈ Purification of PCR Products

After amplification, the PCR sample contains a complex mixture of specific PCR product and residual reaction components such as primers, unincorporated nucleotides, enzyme(s), salts, mineral oil, and probably nonspecific amplification products. Before the specific PCR product can be used in subsequent experiments, it is often necessary to remove these contaminants. The MEGAquick-spin™ Total fragment DNA Purification Kit offers a quick and easy method for purifying the final PCR product. For more information about MEGAquick-spin™ Total fragment DNA Purification Kit, please call iNtRON Technical Service or your local distributor.

◈ Control of Contamination

It is extremely important to include at least one negative control that lacks the template nucleic acid in every PCR setup to detect possible contamination.

A. General physical and chemical precautions

- Separate the working areas for setting up the PCR master mix and DNA handling, including the addition of starting template, PCR product analysis, or plasmid preparation. Ideally, use separate rooms.
- Use a separate set of pipets for the PCR master mix. Use of pipet tips with hydrophobic filters is strongly recommended.
- In case of contamination, laboratory benches, apparatus, and pipets can be decontaminated by cleaning them with a 1/10 dilution of a commercial bleach solution. Afterwards, the benches and pipets should be rinsed with distilled water.
- Prepare and freeze small aliquots of primer solutions and dNTP mix. Use of fresh distilled water is strongly recommended.

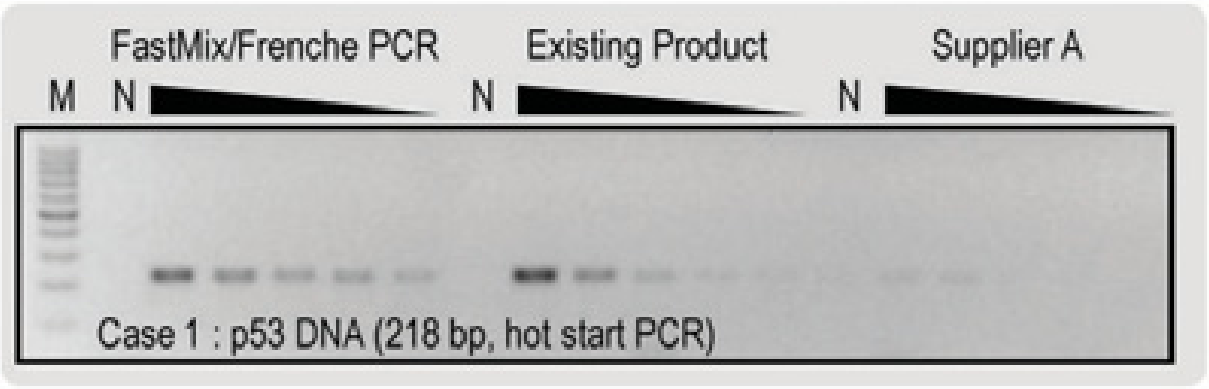
B. General chemical precautions

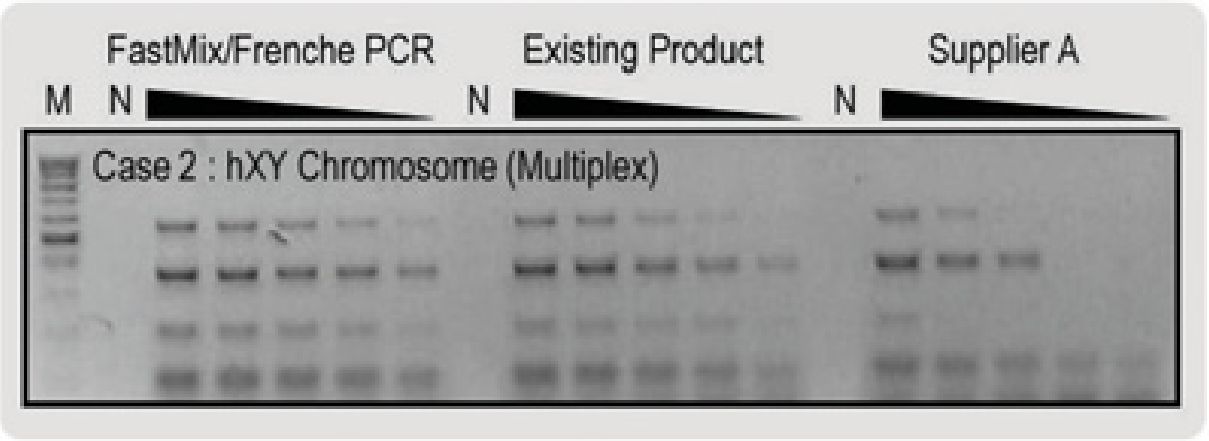
- PCR stock solutions can be decontaminated using UV light. This method is laborious, however, and its efficiency is difficult to control and cannot be guaranteed. We recommend storing solutions in small aliquots and using fresh aliquots for each PCR.
- To prevent amplification of contaminating DNA, treat individual reaction mixtures with DNase I or restriction enzymes that cut between the binding sites of the amplification primers used, before adding the template DNA sample.

EXPERIMENTAL INFORMATION

◈ Improved PCR effectiveness

FastMix / Frenche™ PCR (i-StarTaq) shown enhanced PCR efficiency in term of sensitivity and yield when compared to competing product.

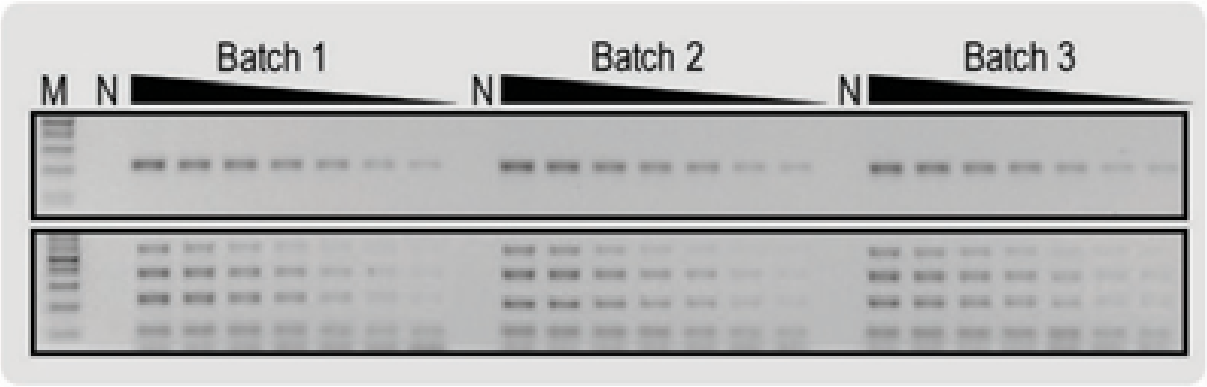




EXPERIMENTAL INFORMATION

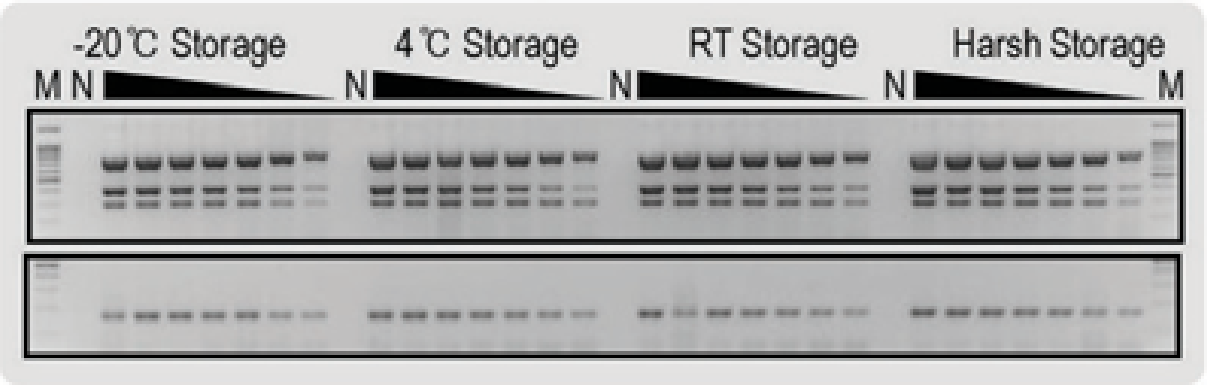
◈ Lot Consistency

FastMix / Frenche™ PCR (i-StarTaq) shown stable batch consistency. The quality of the 3 batch products were confirmed by an equal level.



◈ Stability Test

The amplification activity and stability of FastMix / Frenche PCR Kit was maintained even in the room temperature or extreme circumstance



GLOBAL DISTRIBUTORS

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◈ Molecular Diagnosis

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◈ Molecular Reagent / Molecular diagnosis

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- **High sensitivity, yield and reproducibility**
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