

DIRECT CYSTIC FIBROSIS (CF) DELTA F508 REAL TIME PCR KIT

Cat. No: 14R-30-01

PRODUCT DESCRIPTION

Cystic fibrosis (CF) is a common hereditary disease which affects the entire body, causing progressive disability and often early death and deletion of phenylalanine at residue 508 (delta F508) of the gene is the most common mutation associated with CF. Difficulty in breathing is the most serious symptom and results from frequent lung infections that are treated, though not cured, by antibiotics and other medications. CF is caused by a mutation in the gene for the protein cystic fibrosis transmembrane conductance regulator (CFTR). This gene is required to regulate the components of sweat, digestive juices, and mucus. Although most people without CF have two working copies of the CFTR gene, only one is needed to prevent cystic fibrosis. CF develops when neither gene works normally.

PRINCIPLE OF THE SYSTEM

During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes perfectly to the target DNA. This cleavage results in the fluorescent signal which is monitored by Real-Time PCR detection system. An increase in the fluorescent signal (CT) is proportional to the amount of the specific PCR product.

PRODUCT SPECIFICATION

Each sample should be tested with wild type and mutant real time direct PCR mastermixes. The Kit provides reagents in a ready-to-use direct mastermix format which has been specifically adapted for 5' nuclease PCR using patented SNP analyses. The test system is designed for use with sequence specific primers and probe.

The fluorescence of mutation analysis is FAM. Also each direct mastermix contains an internal control labelled with HEX/JOE dye.

SYSTEM CONTENTS

Reagents	20 rxns	50 rxns
• ΔF508 Wild type Direct PCR mastermix	440 µl	1100 µl
• ΔF508 Mutant Direct PCR mastermix	440 µl	1100 µl
• Control DNA	20 µl	50 µl

* Control DNAs contain plasmid and amplification plots of plasmid DNAs may differ slightly from whole blood sample plots.

STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expiration date on the box label.
- Repeated thawing and freezing (>5X) should be avoided, as this may reduce the sensitivity of the assay.

DNA EXTRACTION

The Direct Kit System is suitable to work from whole blood without DNA extraction step.

Blood samples should be collected in appropriate sterile EDTA or Citrate tubes and can be stored at room temperature.

PROCEDURE

- Leave the Direct mix and controls at RT to melt.
- Mix the melted Direct mix gently by pipetting.
- For each sample, pipet **22 µl Direct mix** with micropipets of sterile filter tips into **White PCR tubes / plate.**
- Turn the peripheral blood tubes for mixing.
- Add **0.5 µl whole blood** into each **White PCR tubes / plate.**
- Optical caps are closed and run with the programme shown below.

PCR PROGRAMME

95 °C	3 Min.	Holding
95 °C	15 Sec.	40x Cycles
62 °C	1 Min.	

Fluorescent dyes are FAM and HEX/JOE

This system can use with;

Bio-Rad CFX96, Opus 96
ABI Prism® 7500/7500 Fast
Roche LightCycler® 480 System
Rotor Gene Q
Mic qPCR Cycler
Long Gene

DATA ANALYSIS

After the run is completed data are analysed using the software with HEX (JOE) and FAM dyes. The below results were studied with Bio-Rad CFX. For positive samples; Amplification plots of internal control and F508 should be close to each other and not exceed 4 Ct (Figure 2).

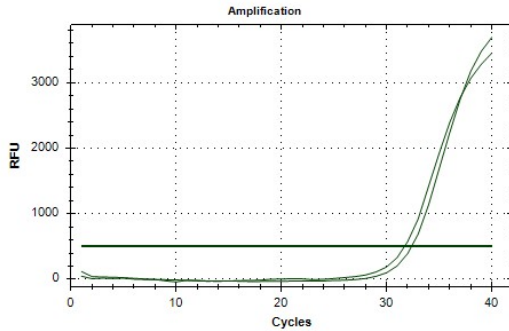


Figure 1: Internal Controls – HEX Dye

Internal control amplification plots must be seen in all wells except NTC and has been labelled with HEX/JOE dye. The CT values of internal controls should be $26 \leq X \leq 36$.

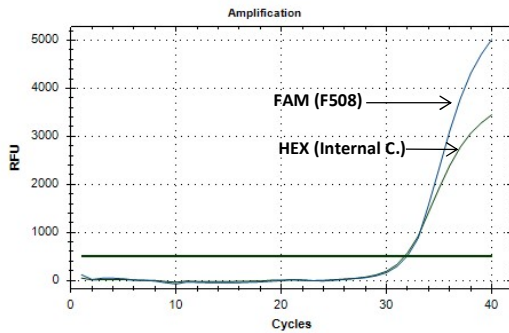


Figure 2: F508 Wild Type Mix – FAM and HEX Dyes.

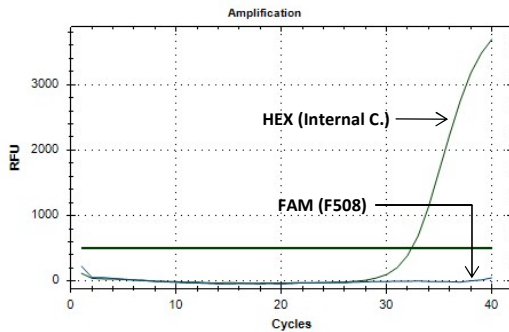


Figure 3: F508 Mutant Mix – FAM and HEX Dyes.

TROUBLE SHOOTING

If internal control doesn't work,

- Absence of sample
- Sample is containing serious PCR inhibitor(s)

Please re-test the sample.

For further questions, please contact us tech@snp.com.tr

CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR mastermixes forgotten at room temperature.
- Thaw PCR mastermix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR mastermix is 12 months. Please check the manufacturing data before use.
- Only use in vitro diagnostics.

- Homozygote wild-type sample gives amplification signal only with wild-type mastermix.
- Heterozygote sample gives amplification signal both with wild-type and mutant mastermixes.
- Homozygote mutant sample gives amplification signal only with mutant mastermix.
- The difference of the CT value wild-type and mutant amplification plots on FAM dye should be ≤ 4 for heterozygote mutant sample.