FISH SPECIES DETECTION FOR BONITO

REAL TIME PCR KIT

Cat. No: 318R-10-01

PRODUCT DESCRIPTION

SNP Fish Species Detection for Bonito Real Time PCR Kit is used for the detection of Bonito *(Sarda sarda)* DNA in raw, processed food and fodder. The Kit provides high sensitivity with a limit of detection (LOD) below the officially required level of 0.1%.

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 quencer 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 isolated DNA should be tested with Bonito Real Time master mix. The system provides reagents in a "Ready-to-Use" master mix format which has been specifically adapted. The test system is designed for use with sequence specific primers and probe for mitochondrial DNA of Bonito.
The fluorescence of species analysis is FAM. Also mastermix contains an internal control labelled with HEX/JOE dye for check PCR reaction.

SYSTEM CONTENTS

Reagents	20 rxns
• Bonito Real Time PCR Master Mix	400 µl
• Bonito Positive Control DNA	30 µl
• Bonito Negative Control DNA*	30 µl

(*) Salmon DNA for check Cross-Reaction.

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 (>3X) should be avoided, as this may reduce the sensitivity of the assay.

DNA EXTRACTION

Fish mitochondrial DNA can be isolated by any Blood Genomic DNA spin column system. 100 mg of sample (meat or processed food) can be used like blood with an additional step. After the **30 min.** incubation step, centrifuge at **13.000 rpm 2 min** and transfer **clear phase** into a new 1,5 ml tube. Than continue with the protocol of spin column system as blood samples after the incubation. Our system optimized according to MN NucleoSpin ® Blood and SNPure® Blood DNA Isolation Kit.

PROCEDURE

- Before starting work, mix the mastermixes gently by pipetting
- For each sample, pipet **20 µl mastermix*** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **5 µl DNA** into each tube**.**
- Run with the programme shown below.

****Master mixes include HotStart Taq DNA Polymerase.***

PCR PROGRAMME

95 °C	3 Min.	Holding
95 °C	15 Sec.	30 Cycles
60 °C	1 Min.	

Fluorescent dyes are FAM and HEX/JOE

If you use;

- ABI Prism® system, please choose **"none"** as passive reference and quencher.
- Mic qPCR Cycler, please adjust gain settings, **"Green Auto Gain"** to **20** and **"Yellow Auto Gain"** to **10.**

This system can use with;

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

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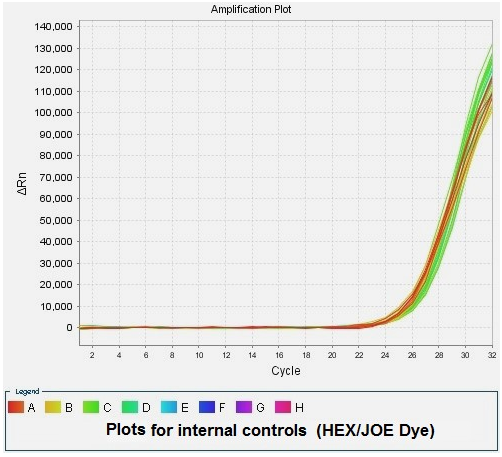
DATA ANALYSIS

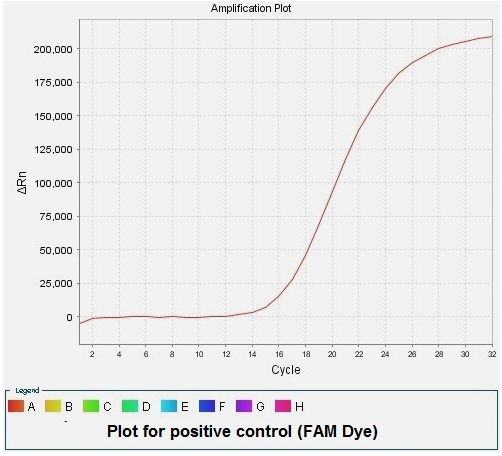
After the run is completed data are analysed using the software with HEX (JOE) and FAM dyes. The below results were studied with ABI 7500.

Internal control amplification plots must be seen in all wells and has been labbelled with HEX/JOE dye. The CT value of internal controls shows **22 ≤ X ≤ 27**.

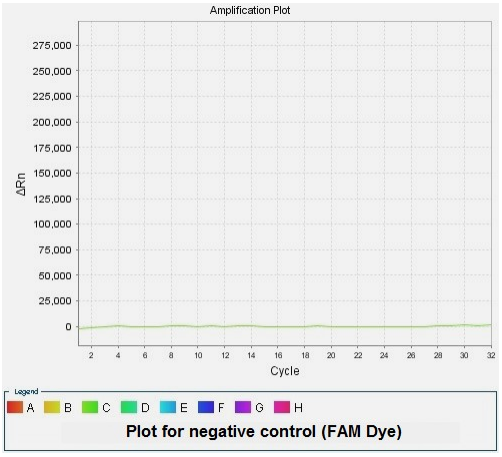


Plots for internal controls (HEX/JOE Dye)

Fish DNA can be analyzed with FAM dye. The CT value of positive sample shows **12 ≤ X ≤ 29**.



Plot for positive control (FAM Dye)



Plot for negative control (FAM Dye)

TROUBLE SHOOTING

If internal control doesn't work,

- Absence of DNA
- Sample is containing DNA inhibitor(s)

If plots start late,

Compare positive control and sample. If there is no problem in positive control,

- DNA quality is not good.
- The amount of DNA is not enough.

Please contact us for your questions. 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.