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ANIMAL SPECIES DETECTION FOR PORK REAL-TIME PCR KIT
Cat. No: 302R-10-01

INTRODUCTION

According to the European Commission Directive 2002/86/EC, food ingredients have to be declared. Animal Species Detection for Pork Real-Time PCR Kit is used for the detection of Pork (*Sus scrofa*) DNA in raw and processed food. The Kit provides high sensitivity with a limit of detection (LOD) below the officially required level of 0.1% [1,2].

INTENDED USE

Animal Species Detection for Pork Real Time PCR Kit can detect the presence of pork material in mitochondrial DNA obtained from processed or unprocessed meat products.

TARGETED USER

For professional use only. Testing should be performed by professionals trained in molecular techniques.

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 (C_T) is proportional to the amount of the specific PCR product [3,4].

PRODUCT SPECIFICATION

Each sample should be tested with Pork Master mix. The kit provides reagents in a **"ready-to-use"** master mix format which has been specifically adapted to 5′ nuclease PCR for SNP analysis. The test system is designed by SNP Biotechnology for use with sequence specific primers and probes.
The fluorescence of Pork analysis is FAM. Also each Master Mix contains an internal control labelled with HEX/JOE dye.

SYSTEM CONTENTS

Reagents	10 rxns	20 rxns	50 rxns
Pork Master Mix	200 µl	400 µl	1000 µl
Pork Positive Control DNA*	30 µl	30 µl	60 µl
Pork Negative Control DNA	30 µl	30 µl	60 µl

Table 1: Kit content

*Since to Control DNAs are synthetic plasmids, amplification plots of synthetic control DNAs may appear slightly different from the sample DNA. Please gently vortex and then spin centrifuge for 1-2 seconds before use the controls.

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 (>4X) should be avoided, as this may reduce the sensitivity of the assay.

SAMPLE COLLECTION

Animal Species Detection for Pork Real Time PCR Kit approved for use with processed or unprocessed meat products.
- Standard precautionary instructions must be followed by all healthcare professionals during the collection and transportation of meat products.
- Meat Products samples should be collected in appropriate containers before delivery to the laboratory.
- Freezing and thawing of samples should be avoided.

DNA EXTRACTION

Animal 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 GeneAll® Exgene™ Blood SV. It is advised to elute DNA with 150 µl elution buffer for better results.

PROCEDURE

- Leave the master mix* and controls at RT to melt.
- Before starting work, mix the master mix gently by pipetting
- For each sample, pipet **20 µl master mix** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **5 µl DNA** into each tube. Please do not pipette DNA before and after addition into well.
- Optical caps are closed, it is recommended to spin the plates/strips at low speed for a short time.
- Run with the programme shown below.

*Master mix include HotStart Taq DNA Polymerase.

PCR PROGRAMME

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

Table 2: PCR Programme

Fluorescent dyes are FAM and HEX/JOE.

<u>This system can be used with the following devices;</u>
- Bio-Rad CFX96
- ABI Prism® 7500/7500 Fast
- Roche LightCycler® 480 System
- Rotor Gene Q
- Mic qPCR Cycler

For other two or more channel Real-Time PCR devices (which can read FAM and HEX/JOE dyes), a trial run is recommended.

<u>If you use;</u>
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**.

Supplied Materials
- White PCR plates/strips with optical covers*

*The PCR Plate/strip tube and caps seriously affect the amplification curve quality. Therefore, white PCR plates/strips and optical caps provided by the manufacturer should be used with the kit.

Required Materials (Not Provided)
- PCR Cabinet
- Vortex Mixer
- Desktop Microcentrifuge (For 2.0ml tubes and PCR strip tubes), plate spin for studies using PCR plates.
- Automated or spin column based DNA isolation Kit
- Disposible powder-free laboratory gloves
- Micropipettes (0.5ml-1000ml)
- Micropipette tips
- Standard laboratory equipments.

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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 Bio-Rad CFX96. The threshold values for both FAM and HEX/JOE dyes were set to 500, based on experiments conducted using the Bio-Rad CFX96 Real-Time PCR system, the GeneAll® Exgene™ Blood SV Isolation Kit, and white PCR strips supplied by SNP Biotechnology. Threshold values may vary depending on the PCR device, DNA isolation kit, and the type or brand of PCR strips/tubes used.

Internal control amplification plots must be seen in all wells except NTC and has been labelled with HEX/JOE dye. The C_T value of internal controls should be **$20 \leq C_T \leq 27$**. These values are optimised according to the GeneAll® Exgene™ Blood SV Isolation Kit and Bio-Rad CFX96 Real-Time PCR Device. C_T values may vary ±2/3 cycle according to the other DNA isolation systems and Real-Time PCR devices (Figure 1).

The presence of amplification plots at the FAM dye should be evaluated as **"Pork Positive".** Amplification C_T values should be **$20 \leq C_T \leq 29$** for positive DNA samples and positive control at FAM dye. These ranges may differ depending on concentration of the content, the PCR instrument, threshold values and tubes used (Figure 2).

If there is no amplification plot at the FAM dye, the sample is evaluated as Pork negative (Figure 3).

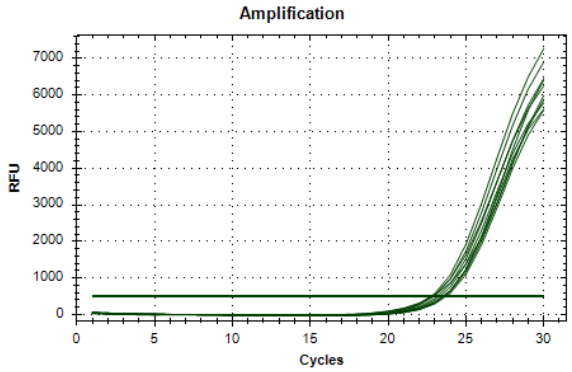


Figure 1: Internal Control plots – HEX/JOE Dye

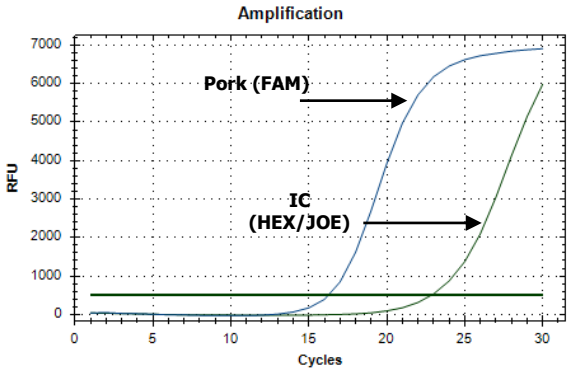


Figure 2 : Pork Positive Sample (FAM and HEX/JOE Dyes)

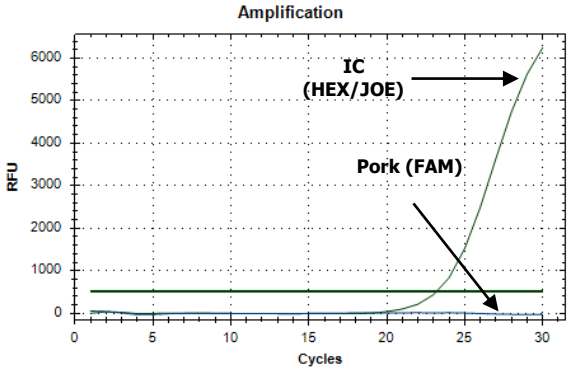


Figure 3: Pork Negative Sample (FAM and HEX/JOE Dyes)

CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR master mixes forgotten at room temperature.
- Thaw PCR master mix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR master mix is 12 months. Please check the manufacturing data before use.
- Can be used for Research Use Only.

DISPOSAL OF KIT

Dispose of it according to the legal regulations of your region

SYMBOLS AND DESCRIPTIONS

REF	Catalog Number	RUO	Research Use Only
LOT	Lot Number	-20 °C	Storage Temperature
	Manufacturer	Σ	Test Quantity
	Fragile		
	Protect from directly sunlight		
	Expiry Date		

Table 3: Symbols and descriptions

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TROUBLESHOOTING PROBLEMS AND SOLUTIONS

Problem	Reason	Solution
Internal control does not work/ low amplification	DNA extraction problems	Repeat test
	DNA extraction problems	• DNA extraction should be repeated. • DNA extraction should be replaced with one of the recommended methods.
	Sample is containing PCR inhibitor(s)	
No target DNA/internal control amplification curves in all wells	Error in temperature/time settings in PCR program	Correct any errors in the temperature/time settings in the PCR Program and repeat the test.
	Sample is containing PCR inhibitor(s)	• DNA extraction should be repeated. • DNA extraction should be replaced with one of the recommended methods.
Low and/or invalid amplification curves of Positive Controls	Stability problems arising from repeated thawing and freezing (>4X)	Repeated thawing and freezing (>4X) should be avoided, as this may reduce the sensitivity of the assay.
	Stability problems arising from unavailable storage conditions.	All reagents should be stored at – 20 °C and dark.
	Bubble formation or pipetting error during pipetting	After adding the master mix and sample, it is recommended to spin the plates/strips at low speed for a short time.
For further questions, please contact us **tech@snp.com.tr**		

Table 4: Troubleshooting problems and solutions

REFERENCES

1. Salmah Orbayinah, Hari Widada, Adam Hermawan, Sismindari Sudjadi and Abdul Rohman. "Application of real-time polymerase chain reaction using species specific primer targeting on mitochondrial cytochrome-b gene for analysis of pork in meatball products". J. Adv. Vet. Anim. Res., 6(2): 260–265, June 2019.
2. Miguel A. Rodrı´guez, Teresa Garcı´a, Isabel Gonza´lez, Pablo E. Herna´ndez and Rosario Martı´n."TaqMan real-time PCR for the detection and quantitation of pork in meat mixtures". Meat Science 70 (2005) 113–120.
3. Yolanda S Lie and Christos J Petropoulos. "Advances in quantitative PCR technology: 5' nuclease assays". Current Opinion in Biotechnology Volume 9, Issue 1, February 1998, Pages 43-48.
4. Luis Ugozzoli and R. Bruce Wallace. "Allele-Specific Polymerase Chain Reaction". A Companion to Methods in Enzymology Vol. 2, No. 1, February, pp. 42-48, 1991.