MeatDetect qPCR Kit Pork (Halal)

Cat#: orb653794 (User Manual)

1. General Information

Accurate identification of animal species, detection of substandard meat and quality control in vegetarian or religiously controlled products (e.g. halal) is essential to ensure a high level of food safety. Therefore, the need for scientifically based species identification is becoming increasingly important. PCR is an excellent method for the analysis of food and feed samples, enabling rapid and accurate monitoring.

Biorbyt MeatDetect qPCR Kit - Pork (Halal) allows the fast and sensitive detection of pork in raw, cooked or processed food products. The kit is designed for use by food and feed producers, food control authorities or analytical laboratories.

2. Intended Use

MeatDetect qPCR Kit - Pork (Halal) is a highly sensitive test system for detection of pork DNA using real-time PCR. Even minimal amounts of pork DNA in food or feed samples are reliably detected. The assay includes an internal positive control (IPC) in the reaction mix to semi-quantify amplification, detect false negative results and exclude the presence of inhibitory substances.

The kit combines simple handling with extremely fast detection of pork in less than 1 hour. All components required for DNA extraction and real-time PCR are included.

3. Kit Contents

Component	Cap	Amount per reaction	24 reactions	96 reactions
10 x Extraction Buffer	yellow	200 µl	1,5 ml	2 x 1,5 ml
qPCR Master Pork	red	18 µl	500 µl	4 x 500 µl
Sample Preparation Tubes	1 tube	12 tubes	48 tubes	
Real-Time PCR Tubes	1 tube	24 tubes (3 x 8-tube strips)	96 tubes (12 x 8-tube strips)	
PCR-grade water	white	180 µl	6 ml	2 x 15 ml

4. Quality Control

Each lot of the Biorbyt MeatDetect qPCR kit - Pork (Halal) is tested against predetermined specifications to ensure consistent product quality.

5. Storage

The kit should be stored at -20°C. Minimize the exposure of the ***qPCR Master*** to light. Repeated thawing and freezing should be avoided as it may reduce assay sensitivity. Short term storage at 4 °C is possible. When stored properly, the kit is stable until the stated expiration date.

6. Safety Information

The kit and all included reagents are intended for *in vitro use only*. The kit is designed for *research use only*.
• The product shall only be used by specially instructed and trained personnel.
• Strict compliance with the user manual is required to obtain optimal PCR results.
• For detailed information, refer to the appropriate material safety data sheet (MSDS).

7. Introduction

The kit has been designed and validated for detection of pork DNA in food and feed samples using real-time PCR technology. In the presence of pork DNA a selected target fragment is specifically amplified and detected by an increasing fluorescence signal in the FAM channel of the real-time PCR cycler. The Internal Positive Control (IPC) is detected in the ROX channel.
All steps of the workflow (sample preparation, assay set-up, PCR cycling) are adjusted to each other and optimized to obtain reliable results within a minimum of hands-on time.

Safety precautions

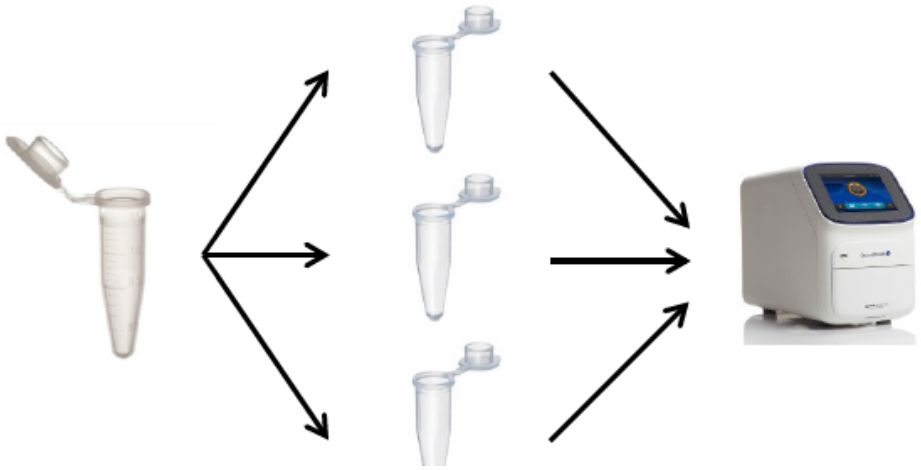
• Kit reagents should be stored in their original containers at indicated temperatures.
• Note the indicated expiry date.
• Store DNA samples separately from kit reagents to minimize the risk of contamination.
• Perform sample preparation in an area separate from PCR assay preparation.
• Pipet sample preparation and PCR assay preparation with sterile filter tips.
• No-template controls should be included in all qPCR runs.

8. Protocol

Before starting

1. Take reagents out from fridge and thaw completely.
2. Switch on the instrument and set all cycling parameters.
3. Vortex all reagents briefly and spin down the material.

Schematic workflow



Sample Preparation	Transfer supernatant	qPCR Assay Preparation	Transfer PCR tubes	qPCR Run
200 µl	2 µl	18 µl Master + 2 µl DNA	FAM signal ROX signal	
5 min	5 min	30 min		

Recommended assay layout

Performing each real-time PCR test in triplets is highly recommended to minimize the risk of detecting false results. Include a triplet of NTCs (negative template controls) in each PCR run to exclude the risk of detecting contaminations from sample preparation or PCR assay preparation.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC	NTC	Test 1a	Test 1b	Test 1c	Test 2a	Test 2b	Test 2c	Test 3a	Test 3b	Test 3c
B	Test 4a	Test 4b	Test 4c									
C												
D												
E												
F												
G												
H												

Sample preparation

1. Dilute ***10x Extraction Buffer*** to ***1x*** with PCR-grade water
2. Aliquot 200 µl of ***1x Extraction Buffer*** into each ***Sample Preparation Tube***

3. Take a small piece (about 2-3 mm in diameter) from meat material or food / feed sample and place it in the tube
4. Mix briefly by tapping or vortexing
5. Incubate for 3 min at room temperature
6. Centrifuge briefly
7. Immediately transfer 2 µl of the ***supernatant*** to the PCR assay (see next step / PCR assay preparation)
8. If required, the supernatant can be stored at -20°C for later use

PCR assay preparation

1. Aliquot 18 µl of ***qPCR Master P****ork* into the required number of ***Real-Time PCR Tubes*** (3 tubes for each sample preparation + 3 NTCs)
2. Add 2 µl of ***Extraction Buffer*** (without extracted DNA) to the tubes for ***Negative Temple Controls (NTCs)*** and close the tubes
3. Add 2 µl of the ***supernatant*** from sample preparation step (extracted DNA) to the tubes for ***qPCR Assay Preparation*** and close the tubes
4. Mix the tubes briefly and spin down to remove bubbles
5. Place the tubes in the qPCR cycler and start the program

Recommended PCR cycling profile

Temperature	Time	Cycles
95°C	2 min	1 x
95°C	15 sec	35 x
60°C	30 sec	

Data collection

- Collect the fluorescence data in the FAM channel for detection of pork DNA
- Collect the fluorescence date in the ROX channel for detection of the IPC (Internal Positive Control) signal

9. Data Analysis

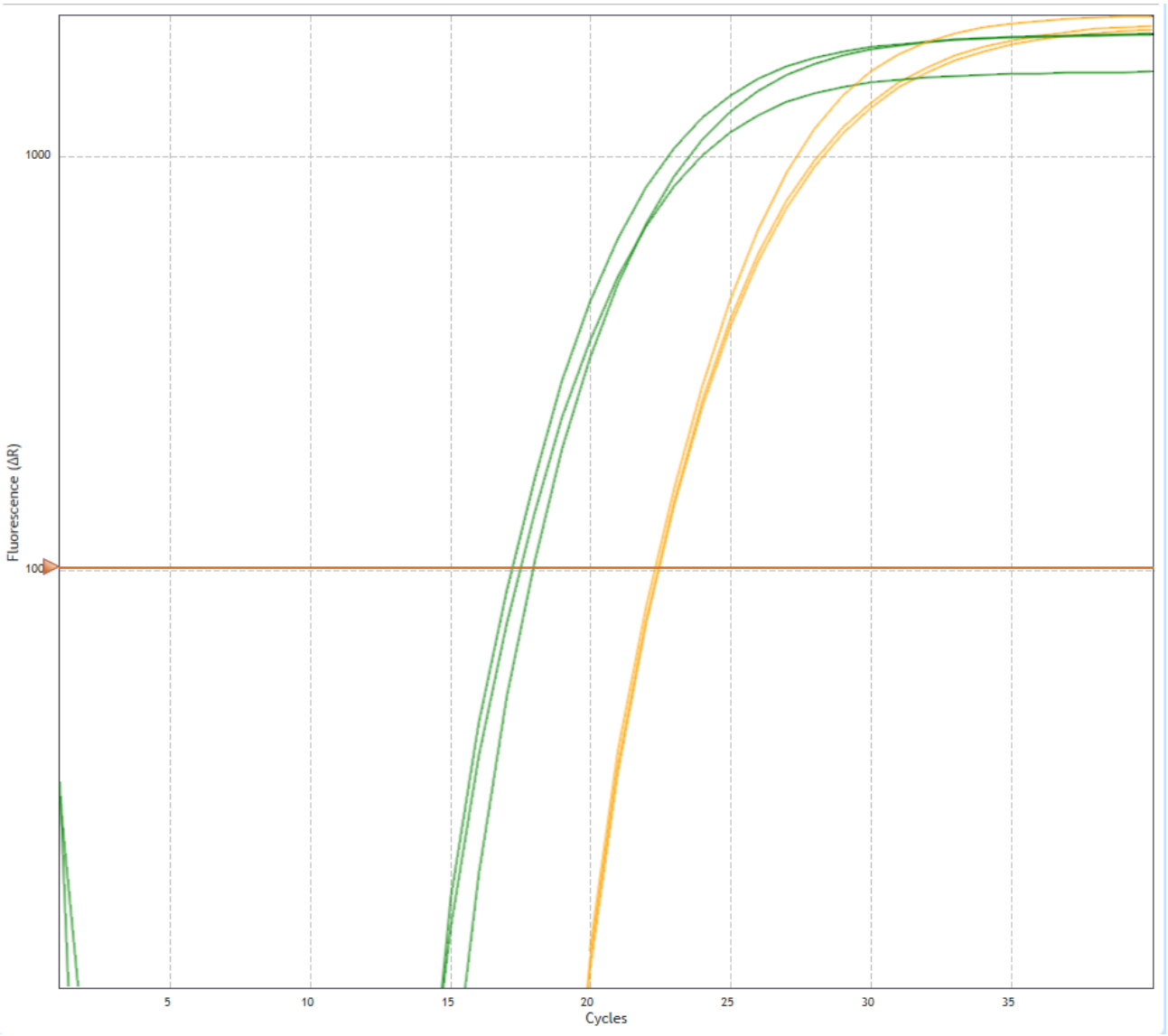
The following results are expected:

FAM fluorescence channel for pork	ROX fluorescence channel for IPC	Result
positive signal ct value 18-24	positive signal ct value 22-28	sample contains pork DNA analyzed food or feed product contains pork meat
low signal ct value 24-30	positive signal ct value 22-28	sample contains only traces of pork DNA analyzed food or feed product contains traces of pork meat

no / negative signal ct value >30	positive signal ct value 22-28	sample does not contain pork DNA analyzed food or feed product is free of pork meat

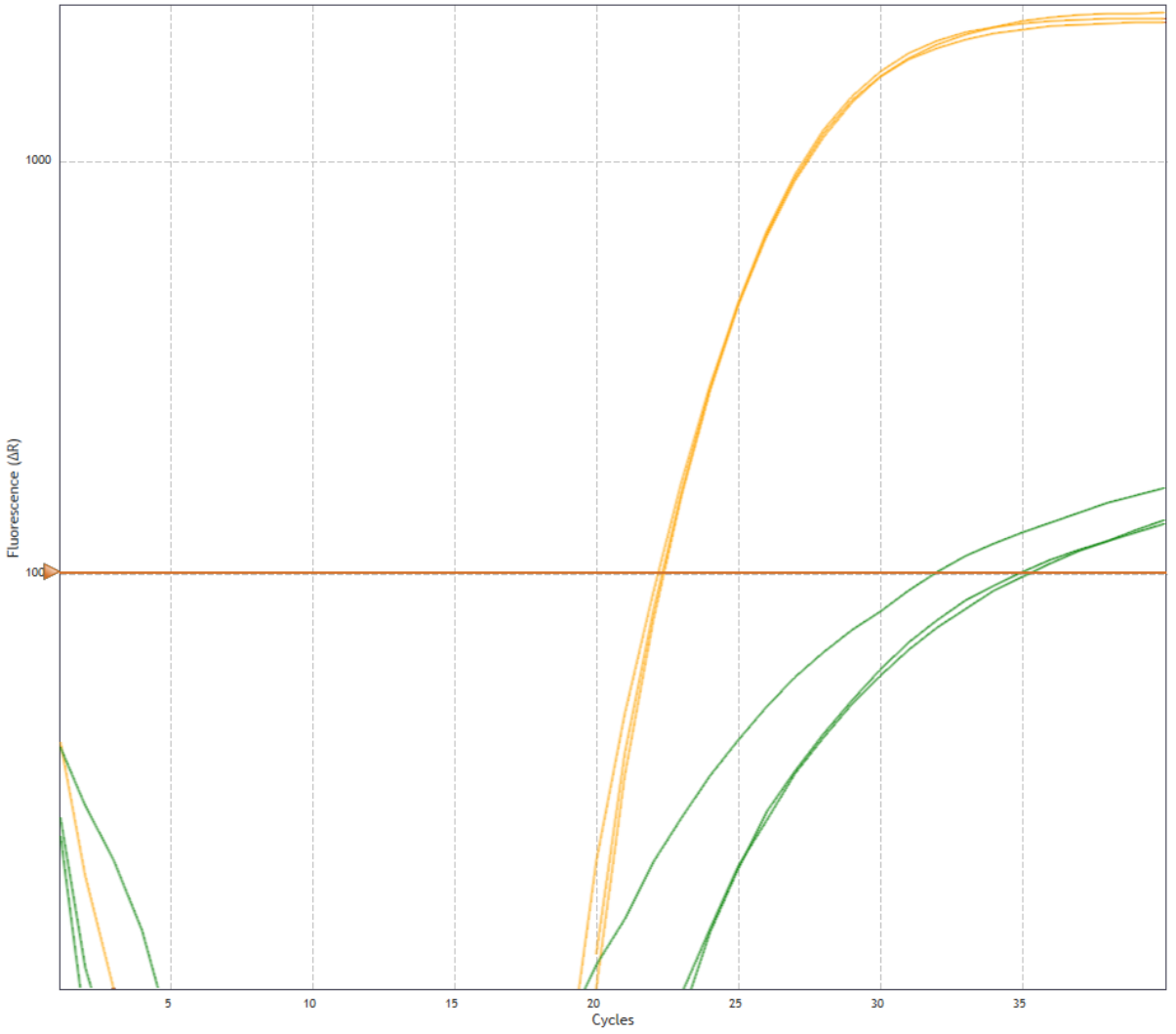
The results correspond with the following amplification plots of the qPCR cycler:

Sample contains pork



Positive pork signal in the FAM fluorescence channel
Positive IPC (Internal Positive Control) signal in the ROX fluorescence channel

Sample contains traces of pork



Low pork signal in the FAM fluorescence channel
Positive IPC (Internal Positive Control) signal in the ROX fluorescence channel

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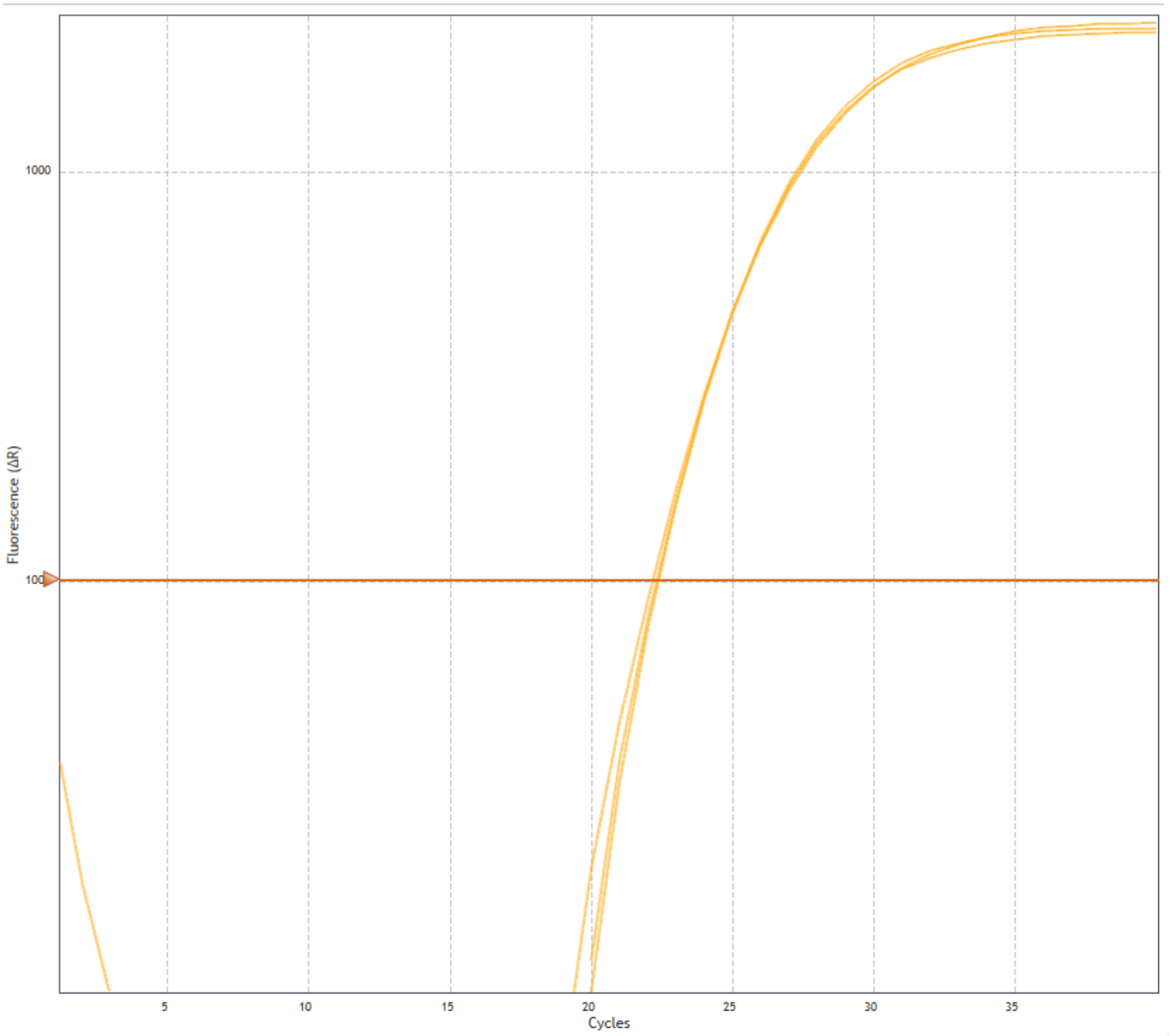
Sample is free of pork



No / negative pork signal in the FAM fluorescence channel
Positive IPC (Internal Positive Control) signal in the ROX fluorescence channel

If no IPC (Internal Positive Control) signal can be detected please refer to chapter 10 for troubleshooting.

10. Troubleshooting

Symptom	Possible reason	Solution
No IPC (Internal Positive Control) signal	Incorrect ***Sample Preparation***	Check the ***Sample Preparation*** step and repeat the test.
Incorrect ***PCR Assay preparation***	Check the ***PCR Assay Preparation*** step and repeat the test.	
Selected fluorescence channel is incorrect	Select FAM channel for pork and ROX channel for IPC.	

Programming of the PCR cycler (temperature / time) is incorrect	Compare the temperature-time profile with the protocol and check correct fluorescence reading.	
Sample contains high amounts of PCR inhibitors	Use lower amounts (1 µl or 0.5 µl instead 2 µl) of ***supernatant*** from ***Sample Preparation***.	
qPCR Master Pork has been exposed to ambient temperature, to bright light or is expired	Check storage conditions and expiration date. Use a new kit.	
Positive FAM signal in NTCs (Negative Template Controls)	Contamination during ***Sample Preparation*** or ***PCR Assay Preparation***	Repeat the complete assay preparation. Make sure to pipet the NTCs first before pipetting the extracted DNA and close the tubes. Perform sample preparation in an area separate from PCR assay preparation. Make sure that the work space is decontaminated in regular intervals.
Extraction Buffer is contaminated with pork DNA	Use a new tube of ***Extraction Buffer***.	
qPCR Master Pork is contaminated with pork DNA	Use a new tube of ***qPCR Master***.	