Jena Bioscience
Building Blocks of Life

Jena Bioscience

Manual

MeatDetect qPCR Kit Pork+Horse (Halal)

	Cat. No.	Amount
	PCR-702-24	24 reactions
	PCR-702-96	96 reactions

IFTA AG
Certified QMS and EMS according to
DIN EN ISO 9001 and DIN EN ISO 14001
Reg.-No.: ICV03597 534 and ICV03597 034 14

Contents

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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.

Jena Bioscience MeatDetect qPCR Kit - Pork+Horse (Halal) allows the fast and sensitive detection of pork and/or horse 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+Horse (Halal) is a highly sensitive test system for simultaneous detection of pork and horse DNA using real-time PCR. Even minimal amounts of pork and/or horse 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 multiplex kit combines simple handling with extremely fast detection of pork and/or horse in 1 hour. All components required for DNA extraction and real-time PCR are included.

3. Kit Contents

Component	Cap	Amount / reaction	24 reactions PCR-702-24	96 reactions PCR-702-96
Direct Extraction Buffer 10x conc. (PCR-534)	yellow	200 µl	1.5 ml	2 x 1.5 ml
qPCR Master Pork+Horse	red	18 µl	500 µl	4 x 500 µl
Sample Preparation Tubes, 500 µl		1 tube	8 tubes	32 tubes
Real-Time PCR Tubes, low profile, 100 µl		1 tube	24 tubes (8-tube strips)	96 tubes (8-tube strips)
PCR-grade Water	white	180 µl	6 ml	2 x 15 ml

Direct Extraction Buffer

Signal word: Danger

Hazard statements:
H314 Causes severe skin burns and eye damage.

Precautionary statements:
P280 Wear protective gloves/protective clothing/eye protection/face protection/hearing protection/....
P301 + P330 + P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P363 Wash contaminated clothing before reuse.
P405 Store locked up.

For further information see Safety Data Sheet

4. Quality Control

Each lot of the *Jena Bioscience* MeatDetect qPCR kit - Pork+Horse (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 *general laboratory 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 simultaneous detection of pork and/or horse DNA in food and feed samples using real-time PCR technology. In the presence of pork and/or horse DNA selected target fragments are specifically amplified and detected by an increasing fluorescence signal in the FAM channel (pork) or JOE channel (horse) 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

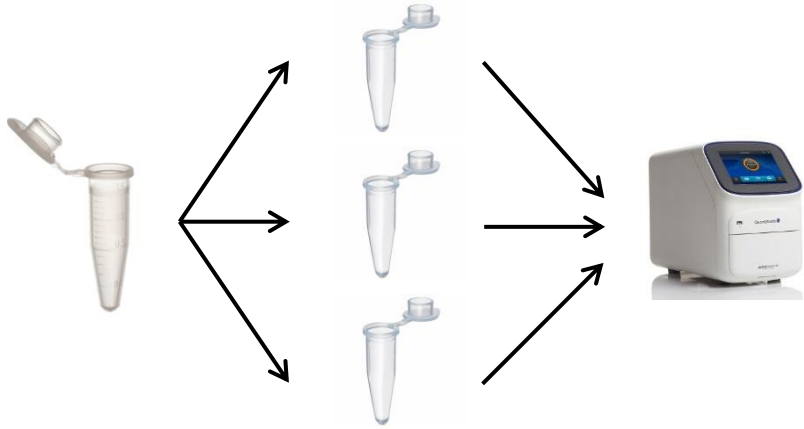
- Kit reagents should be stored in their original containers at indicated temperatures.
- Note the indicated expiry date.
- Store DNA samples separately from kit reagents to minimize the risk of contamination.
- Perform sample preparation in an area separate from PCR assay preparation.
- Pipet sample 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



ample Preparation	Transfer supernatant	qPCR Assay Preparation	Transfer PCR tubes	qPCR Run
200 µl	2 µl	18 µl Master + 2 µl Sample		FAM signal JOE signal ROX signal
5 min				60 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	neg. control	neg. control	neg. control									
B	sample 1	sample 1	sample 1	sample 2	sample 2	sample 2	sample 3	sample 3	sample 3	sample 4	sample 4	sample 4
C	pos. control	pos. control	pos. control									
D												
E												
F												
G												
H												

Sample preparation

1. Dilute ***10x Direct Extraction buffer*** to ***1x*** with PCR-grade water
2. Aliquot 200 µl of ***1x Direct 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 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. se 3 tubes (triplets) for each sample preparation + 3 negative controls (NTC) + 3 positive controls (optional)
2. Aliquot 18 µl of ***qPCR Master Pork+Horse*** into the required number of ***Real-Time PCR 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. For negative controls, add 2 µl of ***1x Direct Extraction Buffer*** (without extracted DNA) to the tubes for ***Negative Temple Controls (NTC)*** and close the tubes
5. Mix the tubes briefly and spin down to remove bubbles
6. Place the tubes in the qPCR cycler and start the program

Positive Controls (optional)

1. If the use of positive controls in the assay set-up is intended, amounts between 10 pg and 1 ng of pork genomic DNA per assay are recommended. Use a triplet of 100 pg of genomic DNA as standard positive control.
2. Dilute ***Pork Genomic DNA (PCR-705, 200 ng/µl, available separately)*** in ***1x Direct Extraction Buffer*** by a factor of 1:20 to obtain a concentration of 100 pg/µl.
3. Dilute ***Horse Genomic DNA (PCR-706, 200 ng/µl, available separately)*** in ***1x Direct Extraction Buffer*** by a factor of 1:20 to obtain a concentration of 100 pg/µl.
4. Prepare a 1+1 mixture of diluted Pork Genomic DNA and diluted Horse Genomic DNA from step 2and 3 (containing pork and horse DNA in a concentration of 50 pg/µl each).
5. Add 2 µl of the mix from step 4 to the tubes containing 18 µl ***qPCR Master Pork+Horse.***

Recommended PCR cycling profile

Temperature	Time	Cycles
95°C	2 min	1 x
95°C	15 sec	40 x
60°C	30 sec	

Data collection

- Collect the fluorescence data in the FAM channel for detection the pork DNA
- Collect the fluorescence data in the JOE channel for detection of horse DNA
- Collect the fluorescence date in the ROX channel for detection of the IPC (internal positive control) signal

9. Data Analysis

The following results for pork DNA detection are expected:

FAM fluorescence channel for pork	ROX fluorescence channel for IPC	Result
no / negative signal ct value > 34	positive signal ct value 20-26	sample does not contain pork DNA → analyzed food or feed product is free of pork meat
positive signal ct value < 34	positive signal ct value 20-26	sample contains pork DNA → analyzed food or feed product contains pork meat

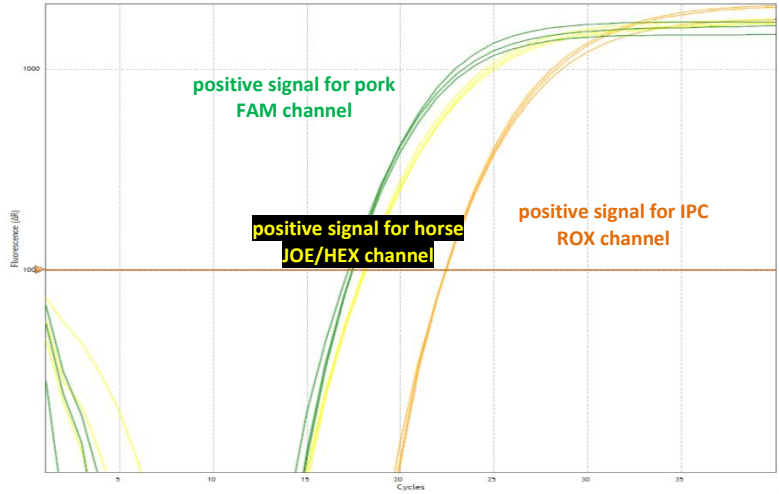
The following results for horse DNA detection are expected:

JOE fluorescence channel for pork	ROX fluorescence channel for IPC	Result
no / negative signal ct value > 34	positive signal ct value 20-26	sample does not contain horse DNA → analyzed food or feed product is free of horse meat
positive signal ct value < 34	positive signal ct value 20-26	sample contains horse DNA → analyzed food or feed product contains horse meat

ct values are evaluated on Thermo Fisher Scientific QuantStudio 3 and QuantStudio 5 real-time PCR cyclers

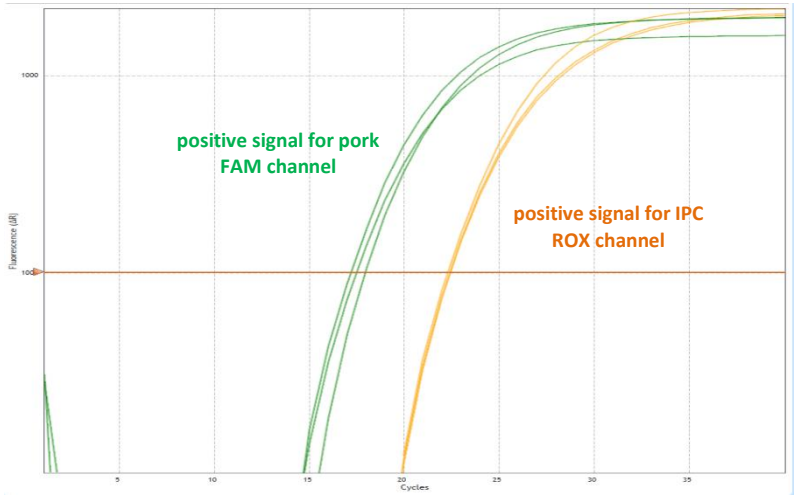
The results correspond with the following amplification plots of the qPCR cycler:

Sample contains pork and horse



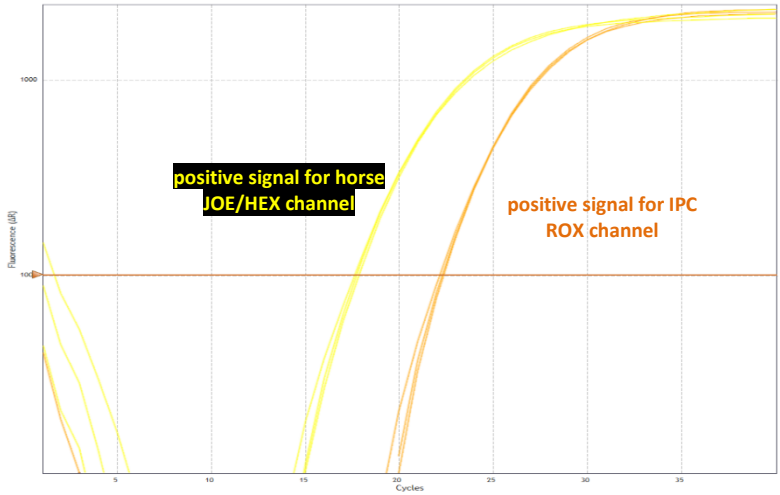
Positive pork signal in the FAM fluorescence channel
Positive horse signal in the JOE channel
Positive IPC (internal positive control) signal in the ROX fluorescence channel

Sample contains pork only (no horse)



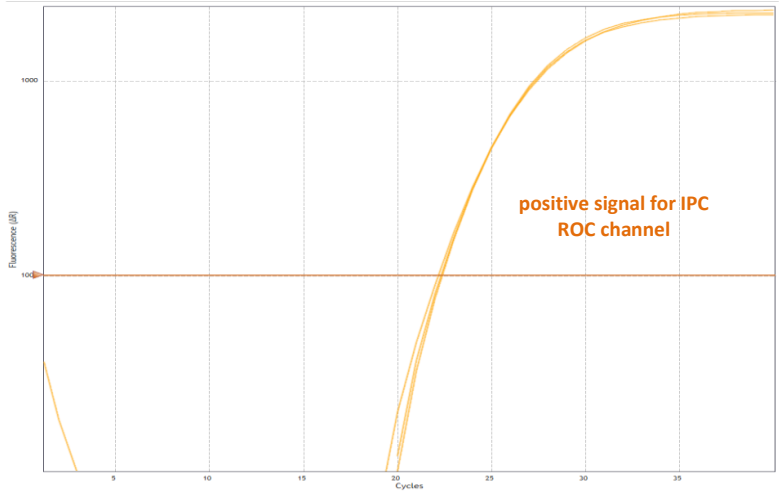
Positive pork signal in the FAM fluorescence channel
No / negative horse signal in the JOE channel
Positive IPC (internal positive control) signal in the ROX fluorescence channel

Sample contains horse only (no pork)



Negative / no pork signal in the FAM fluorescence channel
Positive horse signal in the JOE channel
Positive IPC (internal positive control) signal in the ROX fluorescence channel

Sample is free of pork and horse



Negative / no pork signal in the FAM fluorescence channel
Negative / no horse signal in the JOE channel
Positive IPC (internal positive control) signal in the ROX fluorescence channel

Important: If no IPC (Internal Positive Control) signal can be detected, please refer to chapter 11 for troubleshooting.

10. Detection Limit and Specification

Limit of detection for pork DNA

95% of all samples containing 0.5 pg DNA have been positive tested.
LOD95% = 0.5 pg
The limit of detection for 95% of samples (LOD95%) has been experimentally determined by tested the MeatDetect qPCR Kit Pork (Halal) with Pork Genomic DNA (PCR-705) according to the manual.

Positive tested pork tissues

- Muscle Tissue
- Cartilage
- Skin
- Hoof
- Bone Marrow

Positive tested processed food

- Bacon
- Tinned Meat
- Escalope
- Sausage
- Cotton Swab from kitchen plate

Negative tested species

- Chicken meat
- Maize corn
- Human saliva
- Dog saliva

11. Troubleshooting

Symptom	Possible reason	Solution
ct value of IPC (Internal Positive Control) signal above 26 or no signal detectable	Sample contains high amounts of PCR inhibitors	Prepare a second dilution of the ***supernatant*** from ***Sample Preparation*** in 1x Direct Extraction Buffer in a ratio of 1:10 or 1:100 and add 2 µl to the qPCR Master. Please note that the sensitivity of the test will decrease in the same ratio.
	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.
	qPCR Master Pork+Horse 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 workspace is decontaminated in regular intervals.
	Direct Extraction Buffer is contaminated with pork DNA	Use a new tube of ***Direct Extraction Buffer***.
	qPCR Master Pork+Horse is contaminated with pork DNA	Use a new tube of ***qPCR Master Pork+Horse***.

12. Related Products

MeatDetect qPCR Kit Pork (Halal)
Fast and sensitive detection of pork DNA by multiplex qPCR
PCR-701-24 24 reactions
PCR-701-96 96 reactions

Pork Genomic DNA
Positive control template for PCR
PCR-705 20 µg

Horse Genomic DNA
Positive control template for PCR
PCR-706 20 µg

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