AstraArt-Pork Detection qPCR test Kit

PRINCIPLE

Adulteration of food and cosmetic products with pork derivatives is a major concern in many regions of the world. Detection of pork traces in food and other samples is essential in halal testing for adulteration, as well as for quality control of handling and cleaning processes in production lines.
AstraArt Pork Detection qPCR Test Kit is intended for Real time PCR based detection of unique species-specific sequence of Pork (Sus scrofa) and Internal control present. Even minimal amounts of pork DNA in food, feed samples and cosmetics are reliably detected.

PACKAGE CONTENTS

Description	Specification	Quantity for 100 tests
qPCR Master Mix	qPCR amplification Mix	1000$\mu\ell$ x 1 tube
Primer mix	Pork Primer Probe mix	500$\mu\ell$ x 1 tube
Positive Control	DNA Positive Control	100$\mu\ell$ x 1 tube
Negative control	No Template Control	100$\mu\ell$ x 1 tube
Catalogue Number	AG/PORK/22/02	

STORAGE &STABILITY

- All the reagents should be stored at -20 ℃. Use the reagents within 30 days once opened.
- Completely thaw the reagents before use. Avoid repeated freeze/thaw cycles for reagents.

SAMPLE PREPARATION

- Various samples can be routinely examined for presence of adulterants derived from pork. Extraction of high yield and pure
- genomic DNA need to be carried out using standardized reagents.
- Extracted DNA should be stored at -20 ℃.

ASSAY PROCEDURE:

Nucleic acid isolated from the sample using any validated DNA extraction system. The extracted DNA is directly amplified using the AstraArt Pork Detection qPCR Test Kit on the Real-time PCR Instrument system. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the Real-time PCR Instrument system.

a. Preparation of Real-time PCR reagents: Briefly Centrifuge all the reagents. Prepare the reagents according to the table below. The final volume is calculated by multiplying the number of samples by the volume of each component in Table 1 accordingly.

Table 1: Components of Master mix:

Components	Volume ($\mu\ell$) per reaction
qPCR Master Mix	10
Primer Mix	5
Sample/Positive control/Negative Control	5
Total volume	**20$\mu\ell$**

b. Mix the reaction master mix and spin-down briefly. Aliquot 15$\mu\ell$ of the master mix into each well of 96-well plate and add 5$\mu\ell$ of the sample DNA/Negative control/Positive control accordingly. Seal the plate and spin-down briefly. Run the Protocol immediately on the Real-time PCR instrument with following cycling conditions in Table 2.

Table 2: Cycling Conditions:

Steps	Temperature °C	Time	Cycle
1	95	3 minutes	1
2	95	10 seconds	35
3	53*	25 seconds	
	Pork Target uses FAM and Internal Control HEX (or VIC) channel. *Fluorescence is measured at 53° C.		

Note: Please select "None" in both Passive reference and Quencher.

c. **Interpretation of Results:**

Interpret the values for unknown samples based on the observations as described in the following table. ≤35 Ct of unknown samples should be considered for result interpretation.

Table 3: Conclusion:

	Pork (FAM)	IC (HEX/VIC)	Conclusion
Positive Control	+	+	Valid
Negative Control	-	-	Valid
Sample	+	+	Positive for Pork
Sample	-	+	Negative for Pork
Sample	-	-	Repeat the Protocol with fresh batch of extracted DNA

LIMITATION OF THE PROCEDURE

- Good laboratory practice is essential for proper performance of this assay. Strict compliance with the instructions for use is required for optimal results.
- Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard safety guidelines should be followed.
- A false negative result may occur due to improper extraction or handling.
- Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- The presence of PCR inhibitors may result in lower CT values, false negative or invalid results.

WARNING AND PRECAUTION

- Read the procedure carefully before starting the experiment.
- Wear protective gloves/protective clothing/eye protection/face protection.
- Follow good clinical laboratory practices while handling clinical samples.
- Standard precautions should be followed as per established guidelines.
- Safety guidelines may be referred in safety data sheets of the product

ACCREDITATIONS

 Do not use if package is damaged

 CE mark of Conformity

Refer to the instructions for use

ISO 13485:2016

GMP

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