AstraArt *Horse Spp.* qPCR Kit

PRINCIPLE

AstraArt *Horse Spp.* qPCR Kit is a real-time polymerase chain reaction assay for the qualitative detection of Equus spp. DNA in food and other biological samples. The assay is a combination of the latest advanced buffer chemistry, PCR enhancers and stabilizers along with hot-start Taq polymerase, dNTPs and MgCl2. This assay has been designed for highly reproducible, accurate results in the presence of inhibitors, making it ideal for detection of lowest copy numbers of DNA.

Nucleic acid obtained from samples collected will be amplified with primer probe designed towards **Mitochondrial gene of Target DNA and EIC synthetic control gene.**

PACKAGE CONTENTS

Description	Specification	Quantity 100 tests
qPCR Master Mix	qPCR amplification Mix	1000$\mu\ell$ x 1 tube
Primer Mix	Target specific Primer Probe mix	1000$\mu\ell$ x 1 tube
Positive Control (PC)	Positive Control	500$\mu\ell$ x 1tube
Negative control (NC)	No Template Control	1000$\mu\ell$ x 1 tube
Exogenous Internal Control (EIC)	Extraction control	1000$\mu\ell$ x 1 tube
Catalogue Number	AG/HOR/24/01	

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 REQUIREMENTS

- Specimens collected for Horse DNA investigation should be refrigerated (2 to 8°C) or frozen (-20°C or lower) within one hour after collection.
- If transport exceeds 7 days for the sample to be tested, specimens should be stored at -20°C or lower.
- Longer term specimen storage (>60 days from collection) is recommended at -70°C
- It is ideal to carry out extraction protocol with fresh samples.
- Repeated freezing and thawing should be avoided.
- Transport the specimens at a temperature between 2 to 8°C.

SAMPLE COLLECTION

- Food and other biological samples can be collected.
- Follow universal precautions. All specimens should be considered as potentially infectious and handled accordingly.
- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling specimens and kit reagents.

ASSAY PROCEDURE:

Nucleic acid along with EIC (10$\mu\ell$ per sample) is isolated from the sample using Nucleic acid extraction system and is amplified using the AstraArt *Horse Spp* qPCR Kit on the Real-time PCR Instrument system. In the process, the probe anneals 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	
qPCR Master Mix	10$\mu\ell$
Primer Mix	10$\mu\ell$
Total Volume	**20$\mu\ell$**

b. Mix the reaction master mix and spin-down briefly. Aliquot 20$\mu\ell$ of the master mix into each well of 96-well plate and add 10$\mu\ell$ of the sample DNA/Negative control/Positive control/ Negative extraction 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	2 minutes	**1**
2	95	5 seconds	**5**
3	60	15 seconds	
4	95	5 seconds	**40**
5	60*	15 seconds	
	Equus spp. DNA uses VIC/HEX yellow channel; Exogenous Internal Control uses FAM Green channel ***Fluorescence measured at 60° 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. ≤38 Ct of unknown samples should be considered for result interpretation. The lower limit of detection (LOD) of AstraArt *Horse Spp* qPCR kit is defined as 5 copies/reaction.

Table 3: Determination of CT value:

Fluorescent Channel	CT value	Result
EIC (FAM)	≤ 38	+
Equus spp. DNA (VIC/HEX)	≤ 38	+

Table 4: Conclusion:

results	**EIC (FAM)**	**Equus spp. DNA (HEX/VIC)**	**Conclusion**
Negative Extraction Control	+	-	Valid
Negative Control	-	-	Valid
Positive control	+	+	Valid
Sample	+	+	Positive
Sample	-	+	Positive
Sample	+	-	Negative
Sample	-	-	Invalid run repeat test with fresh sample

LIMITATION OF THE PROCEDURE:

1. AstraArt *Horse Spp* qPCR Kit is used for qualitative detection of Horse DNA from specimens.
2. The specimen to be tested shall be collected, processed, stored, and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
3. Extraction and amplification of nucleic acid from samples must be performed according to the specified methods listed in this procedure.
4. The kit has been validated for the following Real-Time PCR instruments: CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad), CFX96™ Deep Well Dx System (Bio-Rad), CFX96™ Real-Time PCR Detection System (BioRad), CFX96™ Dx System (Bio-Rad), CFX96 Touch Real-Time PCR Detection System (Bio-Rad), QuantStudio Real-Time PCR system (Applied Biosystems).

WARNING & PRECAUTIONS:

- Do not use the product if there is evidence of leakage.
- Adhere to standard procedures and published protocols for sample collection, processing, and disposal.

SYMBOLS:


Do not use if package is damaged


CE mark of Conformity


Refer to the instructions

ISO

GMP

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