PCR Max Ltd™ qPCR test

C.capreolus/C.elaphus/D. dama (Venison)

Venison

Speciation Kit

100 tests

For general laboratory and research use only

Principles of the test

Real-time PCR

This kit provides a method for detecting Venison mitochondrial DNA that may be present in a food sample. The kit is based on the PCR amplification of a unique species specific tag present in the mitochondrial genome of that species. The mitochondrial genome is an ideal target since it has been sequenced for many different species. This allows comprehensive bioinformatics analysis followed by careful design to ensure specific detection of the desired species whilst excluding detection of other related species. Furthermore, since there are multiple copies of each mitochondrial genome within each cell, the detection sensitivity for this kit is up to 100 times greater than that of a test which targets a single copy locus within the nuclear DNA genome.

PCR amplification is detected by means of a hydrolysis probe ("Taqman-style") which is degraded during PCR, releasing fluorescence. The fluorescence trace can be used to both detect and quantify the number of copies of Venison mitochondrial DNA present in the sample.

Sensitivity

Under optimal PCR conditions the kit provides exceptional sensitivity. Priming efficiency is guaranteed >95% and the kit can detect less than 100 copies of the target mtDNA. Assuming 50 copies of mtDNA per cell this equates to a detection sensitivity limit of 1-2 muscle cells within a sample.

Specificity

The kit is designed to specifically detect Venison species that are relevant to the food industry and to give negative detection on other possible meat species.

If you have a query about the detection status of a specific species or sub-species please enquire: enquiry@pcrmax.com

Positive control

The kit provides a positive control template which should be used on every run to prove that your reaction conditions are working correctly. Please note the positive control template poses a significant risk of contamination and should be handled carefully in a separate post PCR environment.

Internal extraction control

When performing DNA extraction, it is often advantageous to have an exogenous source of DNA template that is spiked into the lysis buffer. This control DNA is then co-purified with the sample DNA and can be detected as a positive control for the extraction process. Successful co-purification and real-time PCR for the control DNA also indicates that PCR inhibitors are not present at a high concentration.

A separate primer and probe mix are supplied with this kit to detect the exogenous DNA using real-time PCR. The primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control DNA does not interfere with detection of the Venison target DNA even when present at low copy number. The Internal control is detected through the VIC channel.

Kit Contents

- **Venison specific primer/probe mix (BROWN)**
  FAM labelled
- **Venison positive control template (RED)**
- **Internal extraction control primer/probe mix (BROWN)**
  VIC labelled as standard
- **Internal extraction control DNA (BLUE)**
- **RNAse/DNAse free water (WHITE)**

Reagents and equipment to be supplied by the user

Real-Time PCR Instrument

DNA extraction kit
This kit is designed to work well with all processes that yield high quality DNA with minimal PCR inhibitors.

Lyophilised 2x qPCR Mastermix
This kit is designed to work well with all commercially available qPCR Mastermixes. However, we recommend the use of our own lyophilsed 2x qPCR MasterMix.

Pipettors and Tips

Vortex and centrifuge

Thin walled 1.5 ml PCR reaction tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC on arrival. PCRMax does not recommend using the kit after the expiry date stated on the pack. Once the lyophilized components have been re-suspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and DNA integrity (An internal PCR control is supplied to test for non specific PCR inhibitors). Always run at least one negative control with the samples. To prepare a negative-control, replace the template DNA sample with RNAse/DNAse free water.

Dynamic range of test

Under optimal PCR conditions PCR Max Venison detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

Notices and disclaimers

Trademarks

Reconstitution Protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.

2. **Reconstitute the kit components in the RNase/DNase-free water supplied according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component	Volume
Pre-PCR pack	
Venison primer/probe mix (BROWN)	110 µl
Internal extraction control primer/probe mix (BROWN)	165 µl
Pre-PCR heat-sealed foil	
Internal extraction control DNA (BLUE)	500 µl
Post-PCR heat-sealed foil	
Positive Control Template (RED) *	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

DNA extraction

The internal extraction control DNA can be added either to the DNA lysis/extraction buffer or to the DNA sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control DNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. **Add 4µl of the Internal extraction control DNA (BLUE) to each sample in DNA lysis/extraction buffer per sample.**

2. **Complete DNA extraction according to the manufacturers protocols.**

Real-time PCR detection protocol

1. **For each DNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
Lyophilised 2x qPCR MasterMix	10 µl
Venison primer/probe mix (BROWN)	1 µl
Internal extraction control primer/probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	3 µl
Final Volume	**15 µl**

2. **Pipette 15µl of each mix into individual wells according to your real-time PCR experimental plate set up.**

3. **Pipette 5µl of DNA template into each well, according to your experimental plate set up.**
   To obtain a strong signal, the ideal concentration of DNA is 1-3ng/µl. The concentration should not exceed 5ng/µl. Substitute sample DNA for RNase/DNase free water as a negative control. Substitute sample DNA for positive control template as a positive control.

Amplification Protocol

Amplification conditions using lyophilsed 2x qPCR MasterMix.

	Step	Time	Temp
	Enzyme activation	2 mins	95 °C
40 Cycles	Denaturation	10s	95 °C
	DATA COLLECTION *	60s	60 °C

* Fluorogenic data for the control DNA should be collected during this step through the FAM and VIC channels

Interpretation of Results

Internal PCR control

When used according to the above protocols, CT vales of 28±3 are typical although the exact CT values obtained will depend on a range of criteria including the extraction method employed and the overall experimental work flow. When amplifying a sample with a high mitochondrial genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Qualitative analysis

A positive amplification plot with the test sample indicates the presence of that species within the sample. Since mitochondria are abundant cell components, there are multiple mtDNA copies in each cell and a mitochondria based test is the most sensitive way of detecting species specific DNA. This enhanced sensitivity also means that CT values greater than CT=35 should be regarded as negative test results as this result is equivalent to less than 1 copy of genomic DNA in the sample.

Quantitative analysis (relative expression levels in contaminated meat)

The kit can be used in conjunction with either the PCRMax Universal Meat or Universal Fish Detection kit to provide a quantitative analysis of the level of food sample adulteration. When using 10ng of high quality extracted DNA template, detection of less than 0.1% adulteration is possible. For example by testing a sample with the Universal meat detection kit and the horse meat detection kit, the level of horse meat contamination can be estimated.

Quantitative analysis (relative expression levels in contaminated meat)

Sample A is a beef burger with suspected horse meat contamination
Universal meat CT = 22
Horse CT = 28

Relative expression level of horse = 2^-(28-22) = 0.015

Convert to a percentage = 0.015 X 100 = 1.5% horse in cow

NB CT values >CT=35 should be considered as negative