Primerdesign

Z-Path-SS-B.taurus-Adv

Bos taurus

Cow

Kit version: 1

Target region: Mitochondrial DNA

genesig® Advanced Speciation Kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Product Description

This kit provides a method for detecting Bos taurus mitochondrial DNA in food samples. The kit is based on the PCR amplification of a unique species-specific tag present in the mitochondrial genome. 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 Bos taurus mitochondrial DNA present in the sample.

Specificity

The kit is designed for the in vitro detection of Bos taurus. Specifically, the primers will detect over 95% of sequences available on the NCBI database at the time of last review.

This kit is designed to specifically detect beef species that are relevant to the food industry. The assay may also detect Bos indicus, Bos primigenius, Bos grunniens, Bos javanicus, Bison bonasus (European bison) and Bison bison (American bison).

The dynamics of genetic variation means that new sequence information may become available after the initial design. If you require further information or have a specific question about the detection profile of this kit, then please send an e-mail to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

Quantity	Component	Tube	Cap Colour
1	**Bos taurus specific/Universal animal primer/probe mix (100 reactions)** FAM/VIC labelled		**BROWN**
1	**Bos taurus positive control template**		**RED** (in foil wrapper)
1	**RNase/DNase free water** for resuspension of primer/probe mix		**WHITE**
1	**Template preparation buffer** for resuspension of positive control template		**YELLOW**

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix

This kit is intended for use with oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix.

Pipettors and filter tips

Vortex and centrifuge

1.5 ml microtubes

qPCR plates or reaction tubes

Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and DNA integrity.

Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template.

Principles of the test

Real-time PCR

A target specific primer and probe mix is provided, and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridise to the target DNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5′-dye and a 3′-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

The ‘advanced’ speciation principle

Primerdesign’s advanced speciation kits represent a significant advancement in PCR-based speciation testing. These kits are supplied in a multiplex format enabling both species-specific and ‘universal animal’ detection in a single well. The relative signals produced by each of these tests allows the user to calculate speciation percentages with minimal sample usage. Furthermore, the unique application of a positive control sample as a PCR calibrator allows the user to increase the accuracy of their reported results. This calibration also helps overcome many of the assumptions and hurdles associated with PCR, enabling accurate inter-laboratory testing.

An advanced speciation test allows the user to calculate what percentage of sample is derived from the species of interest, along with the sensitivity of the particular test.

Positive control

The kit contains a Bos taurus DNA positive control sample for the PCR set up. This DNA is used to generate Cq values for both the species-specific and the universal component of the speciation test. The difference between these two values provides an accurate representation of a 100% Bos taurus sample.

Each time the kit is used, at least one positive control reaction must be included in the run. This positive sample demonstrates that both sets of primers and probe are detecting the species of interest. If no amplification is observed for the positive control, the test results are invalid and must be repeated.

Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. It is also known as a No Template Control or NTC.

Endogenous control

One of the functions of the universal meat signal is to serve as an endogenous control, confirming the extraction of a valid biological template. An early universal meat signal indicates the presence of a good yield of animal material. A poor signal indicates that there is an insufficient amount of animal material to perform an accurate speciation test.

Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting is 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/probe mix or template is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the kit components in the RNase/DNase free water supplied, according to the table below.**
   To ensure complete resuspension allow primer/probe mix to rehydrate for 10 minutes at room temperature. Vortex each tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

Component - resuspend in water	Volume
Pre-PCR pack	
Bos taurus specific/Universal animal primer/probe mix (**BROWN**)	110 µl

3. **Resuspend the positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
Bos taurus Positive Control Template* (**RED**)	100 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.

qPCR 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
oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix	10 µl
Bos taurus specific/Universal animal primer/probe mix (**BROWN**)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15 µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Pipette 5 µl of DNA template into each well, according to your experimental plate set up.**
   For negative control wells use 5 µl of RNase/DNase free water (WHITE). For positive control wells use 5 µl of the positive control template (**RED**). To obtain a strong signal, the ideal concentration of DNA is 1-3ng/µl. The concentration should not exceed 5ng/µl. The final volume in each well is 20 µl.

qPCR Amplification Protocol

Amplification conditions using oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix.

	Step	Time	Temp
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

* Fluorogenic data should be collected during this step through the FAM and VIC channels.

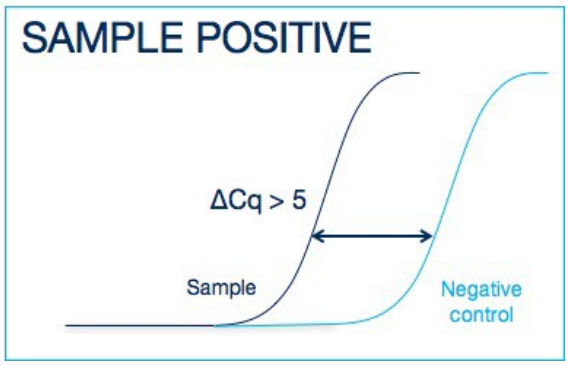
Interpretation of results

Under ideal test conditions the target of interest will give a positive signal, the negative control signal will be negative, and the positive control signal will be positive. In alternative scenarios please refer to the following table for the correct interpretation:

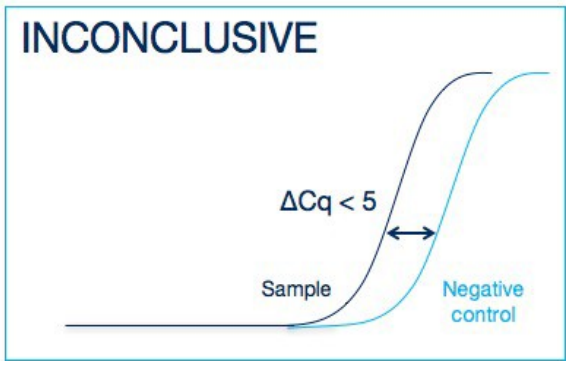
Target (FAM)	Universal signal (VIC)	Positive control (FAM & VIC)	Negative control (FAM only)	Interpretation
≤ 35	**+**	**+**	**-**	**POSITIVE RESULT** calculate species % and check test sensitivity
> 35 or -	**+**	**+**	**-**	**NEGATIVE RESULT**
+	**-**	**+**	**-**	**EXPERIMENT FAILED** due to PCR inhibition
+ / -	**+ / -**	**+**	**≤ 35**	**EXPERIMENT FAILED** due to test contamination
+ / -	**+ / -**	**+**	**> 35**	*
-	**-**	**+**	**-**	**NO ANIMAL DNA DETECTED**
+ / -	**+ / -**	**-**	**+ / -**	**EXERIMENT FAILED**

Positive control template (RED) is expected to amplify before Cq 32 in both the FAM and VIC channels. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

Calculating species %

N.B. A Microsoft Excel applet for automatic % calculation is available free of charge. Contact techsupport@primerdesign.co.uk to request.

Species % = (2^-[(Cq Bos taurus [SAMPLE] – Cq Bos taurus [+ve control]) - (Cq Universal meat [SAMPLE] – Cq Universal meat [+ve control])]) x 100

Worked example: test gives following Cq values:

Bos taurus test on sample: 24.1

Bos taurus test on positive control DNA: 23.5 Universal meat test on sample: 22.2

Universal meat test on positive control DNA: 22.4

Bos taurus% =

(2^-((24.1-23.5)-(22.2-22.4))) x 100 =

(2^ -((0.6) - (-0.2))) x 100 =

(2^ -0.8) x 100 = 57.4%

N.B. In rare circumstances, some samples may produce a speciation % greater than 100. This is usually due to the presence of PCR inhibition affecting the multiplex reaction and should be reported as 100%. If the reported speciation is greater than 400% then the level of PCR inhibition is likely too great for accurate speciation reporting. Samples such as these should be re-extracted with extra washes to remove PCR inhibitors.

Calculating test sensitivity

The sensitivity of a speciation test is dependent on the amount of DNA that has been successfully extracted from a given sample. The genesig® advanced speciation kits have the unique ability to provide information on this sensitivity to empower the user to interpret their data with more precision.

Precise calculations on test sensitivity can be carried out using the Microsoft Excel applet for automatic % calculation that is available free of charge please contact:

techsupport@primerdesign.co.uk

But as a rule of thumb the sensitivity of a given test can be estimated based upon the Cq value achieved from the Universal meat primer/probe.

Universal test Cq	Test sensitivity %
Cq < 19.8	0.01
19.8 ≤ Cq < 23.2	0.1
23.2 ≤ Cq < 26.6	1
26.6 ≤ Cq < 30.0	10
30.0 ≤ Cq ≤ 35.0	Level of animal DNA is too low for accurate speciation testing
Cq > 35.0	Level of animal DNA is too low for analysis to proceed

If the calculated percentage of Bos taurus DNA is greater than the calculated test sensitivity, then the quantitative result is accurate.
If the calculated percentage Bos taurus DNA is less than the calculated test sensitivity, then the quantitative result is not accurate and a qualitative positive result equal to the reported % sensitivity should be reported.

e.g. If your calculated percentage Bos taurus DNA is 1% but the calculated test sensitivity is only 10% then the quantitative result cannot be assumed to be accurate. The qualitative result is still true, however. i.e. the sample does contain Bos taurus DNA. But the percentage can only be assumed less than 10% rather than precisely 1%.

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