Primerdesign™ Ltd

Bubalus bubalis

Buffalo

genesig®

Advanced Speciation Kit

100 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Principles of the test

Real-time PCR

This kit provides a method for detecting Bubalus bubalis 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 Bubalus bubalis mitochondrial DNA present in the sample.

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 Bubalus bubalis DNA positive control sample for the PCR set up. This DNA is used to generate Ct 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% Bubalus bubalis 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.

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.

Specificity

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

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

Kit contents

- **Bubalus bubalis specific/Universal animal primer/probe mix (BROWN)**
  FAM/VIC labelled
- **Bubalus bubalis positive control template (RED)**
- **RNase/DNase free water (WHITE)**
  for resuspension of primer/probe mix
- **Template preparation buffer (YELLOW)**
  for resuspension of positive control template

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

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

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

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. 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.

Primerdesign does not recommend using the kit after the expiry date stated on the pack.

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. 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 genesig Bubalus bubalis detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

Notices and disclaimers

Trademarks

Resuspension 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. **Resuspend the primer/probe mix in the RNase/DNase free water supplied according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in water	Volume
Pre-PCR pack	
Bubalus bubalis 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	
Bubalus bubalis 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 or PrecisionPLUS 2X qPCR Master Mix	10 µl
Bubalus bubalis specific/universal animal primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15µl of each mix into individual wells according to your qPCR 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.

qPCR amplification protocol

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

	Step	Time	Temp
Cycling x50	Enzyme activation	2 min	95 °C
	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

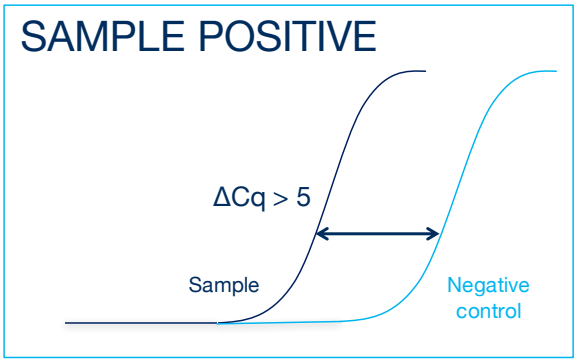
Interpretation of results

Under ideal test conditions the target of interest signal 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:

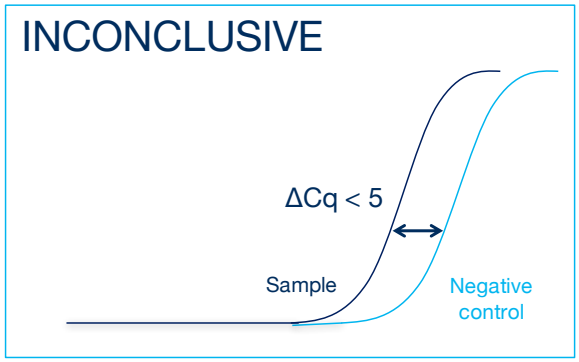
Target (FAM)	Universal signal (VIC)	Positive control (FAM & VIC)	Negative control (FAM only)	Interpretation
+	+	+	-	**POSITIVE RESULT** calculate species % and check test sensitivity
-	+	+	-	**NEGATIVE RESULT**
+	-	+	-	EXPERIMENT FAILED due to PCR inhibition
+ / -	+ / -	+	≤ 35	EXPERIMENT FAILED due to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

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

Worked example: test gives following Cq values:

Bubalus bubalis test on sample: 24.1
Bubalus bubalis test on positive control DNA: 23.5
Universal meat test on sample: 22.2
Universal meat test on positive control DNA: 22.4

Bubalus bubalis% =

(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 then 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 dependant 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. (Contact support@primerdesign.co.uk to request). 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 Bubalus bubalis DNA is greater than the calculated test sensitivity then the quantitative result is accurate.
If the calculated percentage Bubalus bubalis 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 Bubalus bubalis DNA is 1% but the calculated test sensitivity is only 10% then the quantitative result can not be assumed to be accurate. The qualitative result is still true however. i.e. the sample does contain Bubalus bubalis DNA. But the percentage can only be assumed less than 10% rather than precisely 1%