Microbial

FoodReady® BOV

Real-time PCR detection of DNA from cattle (*Bos taurus*)

Introduction

The identification of species in food is becoming a very important issue concerning the assessment of food composition, which is necessary to provide consumers accurate information about the products they purchase. Consumers demand higher protection from falsely labeled meat products for a variety of economic, religious and health reasons, which are enhanced by the recent crisis in the meat sector. In addition, European labeling regulations establish that meat products must be accurately labeled regarding species content (OJEC, 2001), and thus, any ambiguity in the labeling practices of commercial suppliers is unacceptable. The increasing demand for transparency in the food industry has provided a driving force in the development of methods for the analysis of food ingredients

FoodReady BOV is a real-time PCR-based kit that permits detection of bovine DNA for food forensic purposes, mainly authentication and adulteration of foods and meat derivatives.

Presentation

FoodReady BOV is presented in a box containing a pre-mix of all the reagents needed for 48 or 96 determinations. The mix includes an internal amplification control (IAC) which reports for inhibitions in the PCR reactions thus preventing false negative results

- Premix: Bovine DNA test with internal amplification control (IAC)
- Positive control: stabilised *Bos taurus* DNA
- Molecular biology grade H_2O

Technical specifications

FoodReady BOV is a fluorescent hydrolysis probe, real-time PCR based kit, which is intended to detect the presence of DNA from cattle in foodstuff. It can be used for quantitative purposes in a linear range from 20 ng/ul to 20 pg/ul

The primers target a cattle-specific sequence of a mitochondrial gene, found in essentially all eukaryotic cells. Amplicon is short enough (83 pb) to enable the detection of damaged / sheared DNA due to aggressive food processing.

Specificity:	Positive to Bovine (*Bos taurus*). No cross-reaction reported on other animals or plants.
Sensitivity:	20 pg/ul genomic DNA
Limit of detection:	Relative detection level of 0.1 % (see below)
Q-PCR:	
Amplification efficiency:	0.9
Correlation R^2	0.99
Precision ±%:	17.3
Accuracy ±%:	0.2

Use

Extraction and purification of DNA

Use the method of your choice. Many commercial kits are available, which produce good DNA yield from a number of vegetable and transformed products from 20 to 200 mg of samples.

A final DNA concentration of 10 to 50 ng /µl is recommended for optimal performance.

PCR

For each analytical procedure prepare as many tubes as sample to analyze plus two more tubes for controls (positive and negative) by adding 15 µl of FoodReady BOV premix. Add 5 µl of the DNA extract or the positive control provided with the kit. Use 1/10 dilutions if inhibition is observed (see results interpretation below). A blank extraction control should also be run to control cross contamination during the DNA extraction protocol, in particular when processing high number of samples.

Thermocycler program

Cycles	Event	Temperature	Time
1	Polymerase activation	95 °C	10 min
35	Denaturing	95 °C	10 sec
	Annealing and extension ↗	63 °C	1 min

↗ = fluorescence reading. FAM (green channel; excitation at 495 nm; emission at 520 nm) for Bovine detector; JOE/HEX (yellow detector for IAC; excitation at 535 nm; emission at 556 nm)

Results interpretation

Reaction is considered positive whenever an amplification curve for the Bovine detector (FAM) is produced and fluorescence crosses the threshold value established from positive control reaction. Negative results are obtained only when there is no amplification for FAM detector AND there is amplification for IAC (JOE/HEX channel)

FAM	JOE/HEX	Result	Comment
+	+ or –	Positive	A high template quantity can outcompete the IAC amplification
–	+	Negative	
–	–	Inhibition	Dilute or purify the DNA extract

Storage

Store at - 20 °C upon arrival. When frequently used, keep in the refrigerator at 4 °C to avoid repeated freeze-thaw cycles.

Limit of detection

The limit of detection of this real-time PCR system is 20 pg/ul, that is, 100 pg of Bovine DNA per reaction. Therefore, a 0.1% detection limit is achieved for DNA extracts containing ≥ 20 ng/ul of total DNA. For lower concentrations, the following limits of detections should be included in the report

Extract	DNA/reaction	LD
≥ 20 ng/ul	100 ng	0,10%
15 ng/ul	75 ng	0,13%
10 ng/ul	50 ng	0,20%
5 ng/ul	25 ng	0,40%

Important notice 1: DNA fragmentation due to high processing level of some foods may produce negative results in the presence of cattle meat derivatives.

Important notice 2: Results may be expressed either as detected/not detected for sample analyzed (indicating LOD as in table 3) or as percentage of the total DNA. To quantitatively express results, even when using relative (%) units, concentration of the DNA extract from the sample must be determined. Then Cq for Bovine detector must be transformed in to DNA using a calibration curve obtained by serial (1/2) dilution of the standard provided with the kit and expressed as % of the total DNA concentration according to:

$$\%\ Bovine\ DNA = \frac{[DNA]_b}{[DNA]_{total}} \times 100$$

Where $[DNA]_b$ is the bovine DNA concentration obtained after interpolating in the standard curve and $[DNA]_{total}$ is the concentration obtained from the sample DNA extraction.