

# SureFood® ANIMAL identification test kits

- DNA PREP Basic for raw meat and processed products
- Qualitative identification and quantification
- IAAC (Internal Amplification and Animal Control)
- 4plex Screening



## Animal identification

Species specification is of great interest: meat products can be falsified due to incorrect declaration – see horse meat scandal for example. Inferior meat products are falsely declared and thus, a consumer is misled. This may be partly a criminal intent, but the corresponding financial damage as well as the loss of reputation can be highly immense for the distributor.

The required transparency of species identification can be of great interest, both qualitatively and quantitatively in a second step. Quantitative analyses, may detect any falsified declaration of minced meat quantities like “50 per cent pork/beef in minced meat”. Moreover, a second application is the guarantee of religious rules (Halal, Kosher). In this case, no technical threshold of any duty of declaration is tolerated, but especially for the detection of pork in food, a zero tolerance is needed. Consequently, analytical test systems should have a maximum sensitivity.

### Identification

Due to the high DNA concentration in animal cells and the availability of genomic DNA in the nucleus (single copy) and highly conserved DNA in mitochondria (multi-copy DNA), the highly specific analysing of genetic information by real-time PCR is the method of choice. Sub-specification, such as different breeds of one animal species, can be difficult because of no clearly defined DNA specificity. Based on a high stability of DNA, it can be prepared and detected from raw meat (kit S1052: SureFood® PREP Basic) as well as heated, processed meat products provided that DNA is present.

### 4plex screening

Using a corresponding 4-channel qPCR thermocycler with the channels FAM, ROX, VIC/HEX and Cy5, three ruminant can be specifically identified in one reaction as well as an internal amplification control is provided.

In highly purified products such as gelatin, the amount of present DNA has been more or less removed that a reliable measurement might be difficult or no longer be possible.

### Relative quantification

The percentage of specific DNA (e.g. beef) can be detected in present FAM channel with specific hydrolysis probes. In a simultaneous measurement of a serial dilution series, an indication is obtained in DNA copy numbers.

The quantification of the total amount of meat in a parallel measurement shows the quantification of the total amount in DNA copies.

Both values ratioed and multiplied with 100 is the percentage of the species in total proportion of the product's meat content.



Additionally, in the absence of such species it can be observed, whether another species is included in the sample. This analysis is not only of importance for correct labeling of meat and meat products: After an intensive but successful eradication of the BSE crisis and an expected increased use of bone meal in the future, it will be increasingly important to test for the absence of ruminant feed material.

## IAC

The internal control of the Pork SENS PLUS kit (S6117) contains artificial DNA and a corresponding probe within the master mix.

Thus, ensuring a negative signal obtained for pork in the FAM channel for each sample, to guarantee a real negative sample and no possible inhibition. This is important for a religiously motivated analysis ensuring the absence of pork.

## IAAC

In this system, the same channel for the amplification control (HEX/VIC) is used to detect vertebrate DNA. The Cp value results of the internal control ensure a correct amplification and at the same time, it is possible to state whether animal DNA is present or not. Thus the system provides the assurance, that a negative result of the target DNA (measured in the FAM channel) has no significant inhibition (IAC of each sample and the negative control have the same Cp value) on the one hand. On the other hand, the sample may contain animal DNA, if the signal of the internal control of the sample DNA is detected significantly before the signal of the negative control.

## Extraction control and meat/plant detection kit

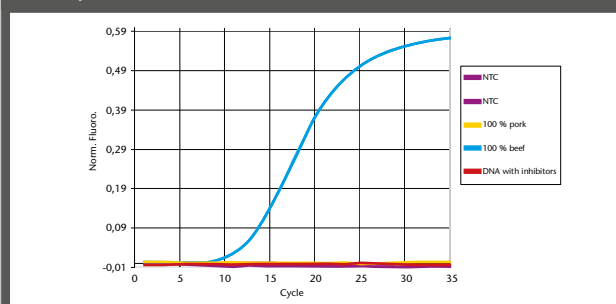
To proof the successful extraction of DNA from samples, a new kit detecting a general plant marker and separately a general marker for land-living vertebrates (including humans) can be used (F4053).

## Vegetarian food

This assay may be also used to detect food samples for the presence of meat. A negative result for meat however does not guarantee per se vegetarian food: The limit of detection is  $> 0$ . The relevant farm animals but not all animals as insects will be detected. Samples of meat origin might be highly processed and the DNA might be degraded.

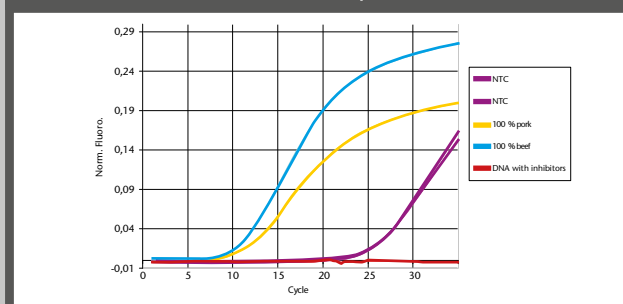


**Fig. 1:** Amplification Plot FAM Channel – Beef specific detection



A first step of analysis is to check if sample(s) in FAM channel shows positive results [Fig. 1]. Here in the beef detection assay, the beef sample (blue line) shows a signal while the known pork sample (yellow line) shows no result. To clarify whether the sample of usually unknown content is really negative (below LOD), switching to the VIC/HEX channel allows a control for inhibition (IAC) and extraction (IAAC) [Fig. 2].

**Fig. 2:** Amplification Plot VIC Channel – Animal DNA detection (example: IAAC run)



The pork sample yellow line shows a signal: no inhibition. The signal is much stronger as the NTC signal: extraction of meat was possible. Conclusion: the unknown sample is negative for beef, no inhibition occurred and meat DNA could be extracted, therefore it is a real negative sample (below LOD).

## SureFood® ANIMAL identification products

Product	Description	No. of Tests	Art.No.
<b>SureFood® PREP</b>			
Basic	DNA preparation of food and feed	100 preparations	S1052
<b>DNA-Extraction control</b>			
SureFast® Animal+Plant control 3plex	Extraction control for plant or animal matrix including internal control DNA (ICD)	100 reactions	F4053
<b>SureFood® ANIMAL ID – qualitative real-time PCR</b>			
4plex Beef/Sheep/Goat + IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6121
4plex Pork/Chicken/Turkey + IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6123
4plex Beef/Horse/Pork + IAAC	Detection limit: 0.5 % depending on matrix and DNA preparation	100 reactions	S6126
4plex Camel/Horse/Donkey + IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6131
Beef IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6113
Horse IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6118
Horse/Donkey IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6119
Pork SENS PLUS	Detection limit: ≤ 0.0001 % depending on matrix and DNA preparation	100 reactions	S6017
Pork IAAC	Detection limit: 0.5 % depending on matrix and DNA preparation	100 reactions	S6114
Water Buffalo IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6117
Chicken IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6115
Turkey IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6116
Cat/Dog IAAC	Detection limit: 0.5 % depending on matrix and DNA preparation	100 reactions	S6112
Rabbit IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6120
Water Buffalo/Beef + IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6130
Poultry IAAC	Detection limit: 0.1 % depending on matrix and DNA preparation	100 reactions	S6125
<b>SureFood® ANIMAL QUANT – quantitative real-time PCR</b>			
Beef	Detection limit: ≤ 5 DNA copies; limit of quantification: 0.04 % depending on matrix and DNA preparation	100 reactions	S1010
Equus	Detection limit: ≤ 5 DNA copies; limit of quantification: 0.01 % depending on matrix and DNA preparation	100 reactions	S1016
Pork	Detection limit: ≤ 5 DNA copies; limit of quantification: 0.04 % depending on matrix and DNA preparation	100 reactions	S1011
Chicken	Detection limit: ≤ 5 DNA copies; limit of quantification: 0.01 % depending on matrix and DNA preparation	100 reactions	S1014



SureFood® PREP Basic



Qualitative real-time PCR Kit

### \* References:

1. Pelin Ulca a, Handan Balta a, İlknur Çağın a, Hamide Z. Senyuva (2013)  
Meat species identification and Halal authentication using PCR analysis of raw and cooked traditional Turkish foods, Meat Science 94, 280-284
2. Yasemin Demirhan a, Pelin Ulca a, Hamide Z. Senyuva  
Detection of porcine DNA in gelatine and gelatine-containing processed food products – Halal/Kosher authentication, Meat Science 90, 686-689