

## Determination of Authenticity and Quality of Chocolate Using Real-time PCR

### Introduction

Chocolate is among the most popular food and flavor types worldwide. Therefore, quality authentication and safety of ingredients and products for human consumption are of major interest for the cocoa industry.

With the emergence of molecular methods and advances in biotechnology, faster, highly sensitive and time-effective methods like real-time PCR are ideal for pathogen detection. In addition, other applications such as the detection of allergens, which can pose major health risks to consumers, or tracing animal DNA e.g., in vegan food products are possible using real-time PCR. The uprise in the allergen-free and plant-based diet demand has exponentially increased and so has the necessity of a rapid yet sensitive method to detect any potential process or origin contamination.

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### Your Benefits

- Rapid detection of allergens, presence of bacterial contamination, and verification of vegan claim
  - Includes optimal DNA extraction methodology using PME from IST Innuscreen
  - Maximum flexibility and ideal real-time PCR signals on qTOWER<sup>3</sup>
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### Methods

DNA from chocolate of 11 different manufacturers was extracted using the PME Food DNA Kit (Cat.# 845-IR-0008050, IST Innuscreen GmbH) and analyzed for various parameters using the following assays:

- innuDETECT Mammalia & Bird Assay (Cat.# 845-IDF-0090096, IST Innuscreen GmbH)
- SureFood® ALLERGEN 4plex Peanut/Hazelnut/Walnut + IAC (Cat.# S3402, R-Biopharm AG)
- SureFast® Salmonella PLUS (Cat.# F5111, R-Biopharm AG)

The qPCR run was performed with an Analytik Jena qTOWER<sup>3</sup> following the instructions in the manuals of the assays used.

### Results

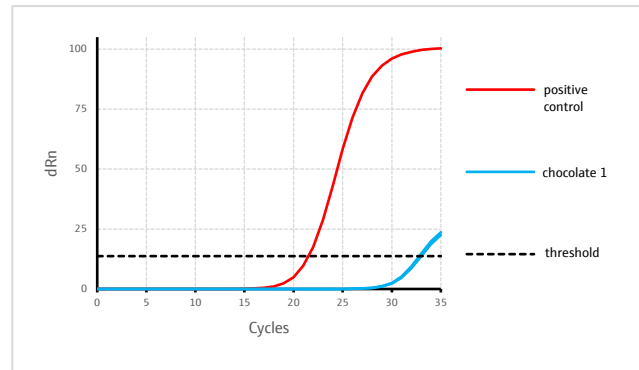
The following results show exemplary data for the different detections. All results can be considered valid as the negative control showed no results and the internal amplification control (IAC) could be detected for all samples.

## Test for the presence of animal DNA

The results of the innuDETECT Mammalia & Bird Assay (IST Innuscreen GmbH) show that the chocolates (chocolate 10 & 11) labelled as vegan do not contain any animal ingredients. Chocolate 1, not labelled as vegan, showed traces of animal DNA.

**Table 1:** Comparison of Ct values of the innuDETECT Mammalia & Bird Assay

Sample	Ct value Mammalia & Bird	Ct value IAC
Chocolate 1	32.9	19.2
Chocolate 10	No Ct	19.53
Chocolate 11	No Ct	19.61
Positive control	21.51	18.55



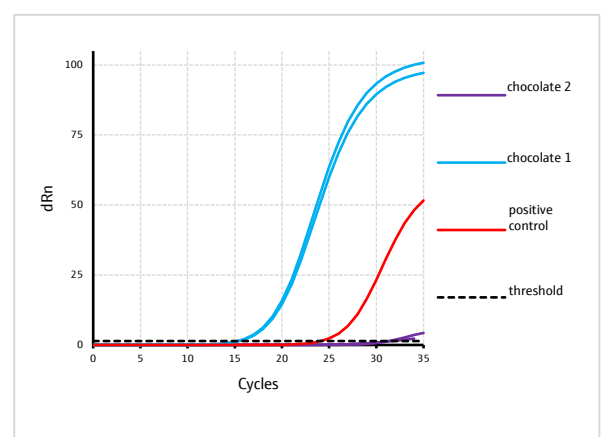
**Figure 1:** Amplification plot of the innuDETECT Mammalia & Bird Assay

## Test for allergenic ingredients

All nut species marked as ingredients could be detected in the respective chocolates. In addition, traces of peanut were detected in chocolate 2. This was also indicated on the list of ingredients.

**Table 2:** Comparison of Ct values of the detection of peanut using the SureFood® ALLERGEN 4plex

Sample	Ct value			
	Peanut	Walnut	Hazelnut	IAC
Chocolate 1	15.19	No Ct	No Ct	21.85
Chocolate 2	31.66	No Ct	No Ct	23.89
Chocolate 8	No Ct	No Ct	19.01	23.8
Chocolate 9	No Ct	No Ct	20.79	23.53
Positive control	23.96	23.1	26.07	23.58



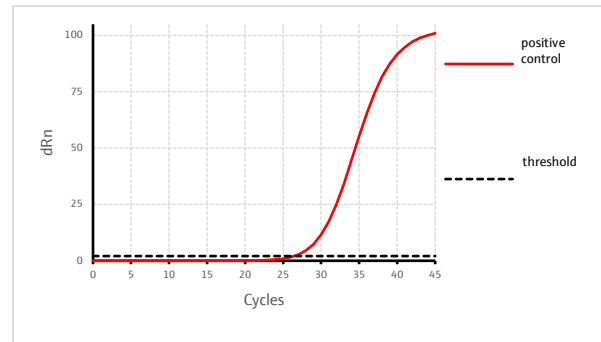
**Figure 2:** Amplification plot of the detection of peanut using the SureFood® ALLERGEN 4plex

## Test for the presence of Salmonella spp.

None of the chocolates showed amplification for Salmonella spp. It can be assumed that the samples are free of this pathogen. The SureFast® Salmonella PLUS (R-Biopharm AG) assay has a detection limit of  $\leq 5$  DNA copies.

**Table 3:** Comparison of Ct values of the SureFast® Salmonella PLUS Assay

Sample	Ct-value <i>Salmonella</i> spp.	Ct-value IAC
Chocolate 1	No Ct	26.81
Chocolate 10	No Ct	26.18
Chocolate 11	No Ct	26.88
Positive control	26.38	25.52



**Figure 3:** Amplification plot of the SureFast® Salmonella PLUS Assay

## Conclusion

Altogether this work demonstrates the suitability of real-time PCR as an excellent tool for ensuring food safety and quality due to its reliability, high accuracy, reduced detection time and targeted detection outcome. The sensitivity of this method is particularly noteworthy, as even traces of potentially allergenic components can be detected. Real-time PCR can also be used to prove that chocolate declared as vegan is certainly vegan, because we did not detect any animal sequences in this chocolate tested.

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