Xpert qDetect Cashew

#GDK30.0100 (100 rxns)
(FOR RESEARCH ONLY)

Product: The presence of allergens in food is an issue of major concern, as reactions triggered by the ingestion of even minimal doses of food allergens varies but could lead to severe potentially lethal anaphylactic shocks. The prevalence of clinical confirmed food allergies indicates that up to 2.5% of adults and up to 8% of children under 3 years of age are affected by food allergens, whereas population surveys have estimated even much higher percentages. According to the European Commission Directive 2002/86/EC, food ingredients must be declared and the most important food allergens must be disclosed on the label. However, for all kind of reasons, this may not be the case, including fraudulent substitution by less expensive ingredients, mislabeling and contamination.

Cashew is one of the allergens listed in this directive. Several methods for Cashew detection that are available are based on protein identification (by means of electrophoretic and/or immunological methods). However, these methods are not reliable for highly processed and heated products due to protein denaturation and degradation. As DNA is much more stable, PCR/qPCR amplification of a species-specific target sequence provide a simple, fast and reliable method for the detection of a target species with high sensitivity and specificity, even in the case of highly fragmented DNA.

The Xpert qDetect Cashew is a kit for the detection by qPCR of DNA from Cashew present in total DNA previously purified from food samples. This kit is compatible with instruments equipped with FAM and ROX channels. The detection limit is 1 pg of Cashew DNA, allowing the detection of as little as 0.001% of target DNA in food samples if 100ng of total DNA (mixed species) is used

Applications: Detection of Cashew DNA in food and other samples.

Contents: The qPCR Detection Kit (#GDK30.0100) for Cashew contains sufficient reagents for 100 qPCR reactions.

Component	GDK30.0100
Cashew Mix A	2x 840 µl
Cashew Mix B	210 µl
Positive Control (Cashew+)	70 µl
Negative Control (Cashew-)	70 µl

Note: This product does not include reagents and other materials required for DNA extraction.

Samples: 2µl of DNA (previously purified from food products using commercially available DNA purification kits according to manufacturer´s instructions or author´s instructions).

Properties: Fast, Easy and Reliable
Low limit of Detection
100% Specificity, determined using DNA from 25 animal and 26 plant species commonly found in same food products, including all the allergens from Directive 2007/68/EC
Compatible with instruments equipped with **FAM** and **ROX** channels

Storage: -20ºC and protected from light for at least 1 year. Minimize repeated freeze/thawing, consider preparation of aliquots.

Prior to use:

General Considerations

In order to prevent erroneous results due to contamination and/or degradation of DNA, it is strongly recommended to have dedicated materials, equipment and area for the DNA extraction, separated from the preparation of PCR procedures. Workflow in the laboratory should be unidirectional, starting with DNA isolation and moving to preparation of PCR amplification and finally to (q)PCR and Detection. Moreover, all molecular biology procedures, including DNA extraction, require qualified personnel to prevent risk of erroneous results and/or inadequate interpretation of results. Disposable gloves must be worn during all procedures. All relevant National and Local Regulations must be met.

Food Sample Preparation

Representative food samples should be prepared conform standard procedures and under GLP conditions.

DNA extraction

DNA is extracted using an appropriate kit (not included) for the extraction of DNA from food samples using the manufacturer´s instructions and/or according to literature following author´s instructions.

Signal Detection

Unambiguous detection of Cashew DNA (and of the internal control) requires a suitable calibration of both FAM and ROX channel. Please refer to the manufacturer´s instruction of the real time PCR cycler.

qDetect - Basic Protocol

1. **Mix for each qPCR reaction**:

Component	Volume
Cashew Mix A	16 µl
Cashew Mix B	2 µl

In order to minimize risk of contamination, reagent loss and improve pipetting accuracy, we recommend to prepare a mastermix for multiple samples (N), always including a negative control, and a positive control, by mixing all components (N+2), except template DNA (nor control DNA), dividing the mixture equally into each PCR tube (18 µl each), briefly spin tubes (or tap down) and then add 2 µl template DNA or control DNA directly in the mixture.

2. **Set-up qPCR cycling**:

Nº cycles	Temp	Time	Acquisition
1x	50ºC	2 min	No
1x	95ºC	5 min	No
45x	95ºC	30 sec	No
	60ºC	30 sec	Yes
	72ºC	30 sec	No

After an initial cycle of 2 min at 50ºC and 5 min at 95ºC (Enzyme activation and denaturation of template), cycle 45 times for 30 seconds at 95ºC, 30 seconds at 60ºC and 30 seconds at 72ºC. Acquire data for the detection of Cashew DNA on the **FAM** channel. Probe to detect specific amplification of the internal control, which is included in Cashew Mix A and which is amplified simultaneously with the target DNA, should be detected in the **ROX** channel.

Results

Controls
In order to validate the assay, controls must have the following results. If the signal of one of the controls does not match, the whole experiment, including all samples, must be repeated.

Control	FAM channel	ROX channel
Negative Control	Ct=N/A	positive
Positive Control	positive	unimportant*

N/A = Not applicable (signal below threshold).
*unimportant: is expected to be positive, however, if negative but other controls match expected results, this makes no difference

Samples
For each sample, there are 4 possible outcomes, as summarized in the table below.

FAM channel	ROX channel	Result
positive	positive	positive
positive	Ct=N/A	positive
Ct=N/A	positive	negative
Ct=N/A	Ct=N/A	inhibition*

*) in case both Cashew (FAM channel) and Internal Control (ROX channel) have signals below threshold, but all the controls resulted in signals as expected, the sample must be retested, as the qPCR reaction was inhibited. Inhibition often is the result of a too high DNA concentration and therefore it is recommended that retesting should be carried out with a 10-fold dilution of the original DNA sample.