Fact Sheet

Detection of Residues of Allergens in Foodstuffs by Polymerase Chain Reaction (PCR)

Method Summary

DNA is extracted and purified from the sample in order to carry out the test. The DNA must be separated from the high levels of lipids, proteins, carbohydrates and other contaminants present in food at high levels, and which would otherwise affect or block (inhibit) PCR.

The sample is first mechanically homogenised and is then incubated with a buffer containing detergents, salt, chelating agents and proteinase K. This extraction buffer breaks-down cellular material and releases the DNA into solution. The sample is incubated with extraction buffer for at least two hours at an elevated temperature. The resulting lysate is centrifuged to remove debris and treated successively with solvents to remove the bulk contaminants mentioned above. Fine purification is achieved using a commercial DNA purification system, which involves the binding and washing of DNA on a silica membrane mounted on a spin column.

The purified DNA is eluted into purified water and assessed for yield and purity by 260/280nm UV spectroscopy. The DNA preparation is then diluted if necessary and analysed by PCR. Primers that identify the DNA sequence to be amplified determine the specificity of the PCR reaction. The PCR primers used in the allergen test are specific to various species of allergen. All of the primers have been designed to amplify small DNA fragments in order that traces of allergen DNA can be detected in highly-processed matrices. The PCR system has been tested against all other allergen types and various samples of plant and animal DNA with no cross-reactions detected. PCR is carried out in parallel with the specific allergen reference DNA and an array of negative controls (see below). In a separate PCR reaction, the sample is spiked with bacterial DNA and PCR is carried out using primers specific to the bacterial system. A product from this reaction demonstrates that all potential PCR inhibitors have been removed from the DNA preparation.

After PCR cycling, the reactions are mixed with loading dye and separated by polyacrylamide gel electrophoresis. After electrophoresis, the gels are stained with ethidium bromide and then photographed on a Gel Documentation system.

In order to classify a sample as positive, the sample must produce a PCR product of equal molecular weight to the allergen reference DNA. Other quality control criteria are:

- All stages of the analysis detailed above are conducted in physically separated laboratories containing dedicated equipment and reagents to minimise the risk of sample contamination
- The sample is analysed in duplicate. A positive result must be observed in each duplicate.
- A PCR and extraction reagent blank is included in each run. These must show no evidence of contamination in order to report the results.
- If the sample is negative, the inhibitor check PCR must return a positive result to show that the sample does not contain inhibitors.
- The relevant allergen reference DNA is diluted to verify the sensitivity of the test. The diluted allergen reference DNA must produce a duplicate positive result in order to confirm the sensitivity of the test.

For further information contact Customer Services
Phone: +44 (0) 118 9184076 Email: enquiries@rssl.com www.rssl.com