Why test for Food Allergens?

- Brand Name Protection
- Prevent Costly Product Recalls
- Ensure Consumer Confidence
- Duty of Care
- Comply with Product Labelling Requirements.

Allergens may occur unintentionally in foods for several reasons including:

- Cross-contamination of ingredients
- Food preparation errors
- Improper cleaning of equipment

Kits available:
- Almond, Buckwheat, Beta-Lactoglobulin, Casein, Crustacean, Egg, Gliadin, Hazelnut, Lupin, Mustard, Peanut, Sesame, Soy

Product Code:  ESMUS-48

For the detection of Mustard Seed Protein Residues in Food Products and Environmental samples.

Intended Use
The ELISA SYSTEMS Mustard Seed Protein Residue assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen food products for the presence of mustard seed protein material.

Background:
The European Directive 2003/89/EC includes mustard as a declared food allergen and as such, mustard must be included on food product labels under the appropriate labelling laws regarding food allergens. Mustard is widely used as a flavouring agent, as well as for its texture-enhancing and emulsification capacity in various food preparations, particularly with salad dressings and meats.

This assay has been shown to detect the seed proteins from: White Mustard (Brassica alba - sometimes known as Sinapis alba or Yellow Mustard), Black Mustard (Brassica nigra), and Brown Mustard (Brassica juncea).

Interpretation of Results:
This assay is based on comparison to soluble mustard seed protein.

Please note: A special extraction solution is required for samples containing Polyphenols, including: Wine, Herbs, and Tannins.
(Product code:  ESADDSOL)

Controls Supplied
- 0, 1.0, 2.5, 5.0 and 10.0 ppm (mg/kg) Soluble Mustard Seed Protein.

How the ELISA SYSTEMS Mustard Seed Protein Residue test works:

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample is added</td>
<td>Antigen-Antibody Complex</td>
<td>Coloured End-Point</td>
</tr>
</tbody>
</table>

The test sample is added and if Mustard Seed residue is present, it will bind to the specific antibodies.

Enzyme-labelled Conjugate is added and binds to the captured Mustard Seed residue to form a “Sandwich”.

TMB Substrate is added, which is converted in the presence of the Enzyme Conjugate to form a blue colour if Mustard Seed residue is present in the sample. A yellow colour is formed once Acid is added to stop the reaction.

Total test time is approximately 80 minutes on extracted samples. (Incubation times of 30, 30 and 15 minutes each.)
Food Allergen Residue ELISA Protocol

Add 100 microlitres of Standards and Samples to their allocated Antibody-coated wells. Mix all wells for 10 seconds by gentle shaking on a flat surface. **Incubate for 30 minutes.**

Dump liquid from wells. Wash wells thoroughly five times with wash buffer. Tap wells firmly onto absorbent paper towel.

Add 100 microlitres of the Green Conjugate Solution to each well. Mix all wells for 10 seconds by gentle shaking on a flat surface. **Incubate for 30 minutes.**

Dump liquid from wells. Wash wells thoroughly five times with wash buffer. Tap well firmly onto absorbent paper towel.

Add 100 microlitres of the Substrate Solution to each well. Mix all wells for 10 seconds by gentle shaking on a flat surface. **Incubate for 15 minutes.**

**DO NOT WASH**

Add 100 microlitres of the Stop Solution to each well. Mix all wells for 10 seconds by gentle shaking on a flat surface.

Read results visually, comparing with the colour of the Standards. The results can be read on a microplate/strip reader. Results must be read within 30 minutes.

Interpretation of Results
Interpretation is based on the suggested extraction/dilution protocol. Results are for screening purposes. All results should be interpreted as part of a HACCP plan for Food Allergens. Any sample returning a positive result should be regarded as a presumptive result and confirmation or further testing should be performed.

<table>
<thead>
<tr>
<th>Product Code</th>
<th>No. of Wells</th>
<th>Max No. of Tests</th>
<th>(Depending on the number of samples and controls per run)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESMUS-48</td>
<td>48</td>
<td>46</td>
<td>as a screening test (a multichannel pipette must be used to achieve maximum sample numbers)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30+</td>
<td>as Quantitative test</td>
</tr>
</tbody>
</table>

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