Mustard ELISA Kit

Enzyme Immunoassay for the quantitative determination of Mustard in food

Catalog number: ARG80807

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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MANUFACTURED BY:

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INTRODUCTION

Mustard belongs to the Brassica plants. With about 30-35% the fraction of proteins in mustard seed is very high. Some of these proteins are known for being aller-genic, such as Sin a 1 and Bra j 1. These proteins are predominantly heat resistant making them stable to different production processes. In addition to brown mustard (Brassica juncea) and black mustard (Brassica nigra) primarily yellow mustard (Sinapsis alba) is used as an ingredient in many foods and food preparations. For mustard allergic persons hidden mustard allergens in food are a critical problem. Already very low amounts of mustard can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, mustard allergic per-sons must strictly avoid the consumption of mustard or mustard containing food. Cross-contamination, most-ly in consequence of the production pro-cess, is often noticed. The sausage production process is a repre-sentative ex-ample. This explains why in many cases the existence of mustard residues in food cannot be excluded. For this reason sensitive detection systems for mustard residues in food-stuffs are required.

The Mustard ELISA represents a highly sensitive detection system for yellow mustard and is particularly capable of the quantification of residues in sausage, dressings, soups, cheese and mixed herbs. Due to high cross-reactivity the test is also suitable for the detection of brown mustard and black mustard.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody directed against mustard proteins is bound on the surface of a microtiter plate. Mustard containing samples or standards are given in-to the wells of the microtiter plate. After 20 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugated second antibody directed against Mustard is given into the wells and after 20 minutes of incubation the plate is washed again. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of Mustard is directly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-antibody Conjugate	15 ml (ready to use)	4°C
Standards (0, 2, 6, 20, 60 ppm)	5 X 2 ml	4°C
10x Extraction and sample dilution buffer	2 X 120 ml	4°C
10x Wash Buffer	60 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer, Extraction Buffer and Sample diluent buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample. Hazelnut proteins adhere very strongly to different surfaces. In certain cases they can resist a common dishwasher cleaning. To identify possible cross-

contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

The following sample preparation should be applied for all kinds of samples:

1. To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
2. 1 g of the homogenized mixture is suspended in 20 mL of pre-diluted extraction buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at 60°C. To ensure good homogeneity, the samples should be shaken every two minutes.
3. The samples are centrifuged for 10 minutes at 2500 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. 100 µL of particle-free solution are applied per well. If the results of a sample are out of the measuring range, further dilution with the pre-diluted extraction and sample dilution buffer is necessary. The additional dilution has to be considered when calculating the concentration.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.
- **1X Extraction and Sample diluent buffer:** Dilute 10X Extraction and Sample diluent buffer into distilled water to yield 1X.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of standards and samples in duplicate into wells.
3. Incubate for **20 minutes** at **RT**.
4. Aspirate each well and wash, repeating the process 2 times for a total **3 washes**. Wash by filling each well with 1X wash buffer (**350 µl**) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µl** of HRP-Antibody Conjugate into each well. Incubate for **20 minutes** at **RT**.
6. Aspirate and wash well as step 4.
7. Add **100 µl** of TMB mixture to each well. Incubate for **20 minutes** at **room temperature** in dark.
8. Add **100 µl** of Stop Solution to each well.
9. Read the OD with a microplate reader at **450 nm** immediately. It is recommended read the absorbance within 30 min after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

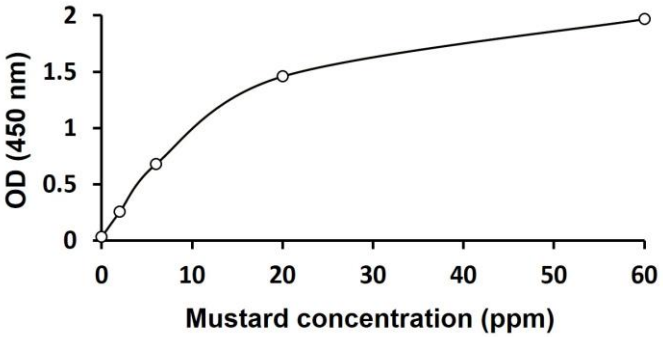
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of the Mustard test is 1 ppm.

The limit of quantification (LOQ) of the Mustard test is 2 ppm.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

Specificity

The following cross reactions were determined:

Milk	Corn	Radish
Egg	Buckwheat	Cabbage
Pork	Soy	Pepper
Beef	Sesame	Curcuma
Chicken	Fish (Cod)	Cayenne
Wheat	Pea	Clove
Rye	Bean	Nutmeg
Oats	Carrot	Cinnamon
Barley	Leek	Dill
Rice	Celery	Thyme
Caraway	Fennel	

The following cross-reactions were determined:

Horseradish	0.0007%
Garden cress	0.0009%
Garden cress (seed)	1.5%
Rape (seed)	15.5%
Radish (seed)	31.2%
Cabbage (seed)	29.2%
Brown mustard (seed)	26.5%
Black mustard (seed)	32.5%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 8% and the CV value of inter-assay precision was 12%

Recovery

Sausage	98%
Salad dressing	76%
Instant soup	80%
Canned soup	96%
Cheese	89%
Mixed herbs	78%