



DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com



See external label



2°C-8°C



96 tests



5141-8

Enzyme Immunoassay for the Quantitative Determination of Lupine/Lupin in Food

LUPINE

Cat #5141-8

Sensitivity	0.2 ppm
Recovery (spiked samples)	98%-113%
Incubation Time	60 min

GENERAL INFORMATION

Lupine/Lupin belongs to the legume family. With about 35-45% the fraction of proteins in lupine seed is very high. Some of these proteins, predominantly those in the α -, β - and γ -Conglutin fractions are known for being allergenic, due to cross-reaction to allergens of soy and peanut. Because of its convenient nutritional properties the use of lupine flour as a food ingredient is highly increasing. Lupine is often used in dietary products, especially for milk allergic people, vegetarians and patients with celiac disease. In food production three (sweet) lupin species are commonly used: *Lupinus albus*, *Lupinus angustifolius* and *Lupinus luteus*.

For lupine allergic persons hidden lupine allergens in food are a critical problem. Already very low amounts of lupine can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, lupine allergic persons must strictly avoid the consumption of lupine containing food. Cross-contamination, mostly in consequence of the production process, is often noticed. This explains why in many cases the existence of lupine residues in food cannot be excluded. For this reason sensitive detection systems for lupine residues in foodstuffs are required.

The **DAI Lupine ELISA** represents a highly sensitive detection system for lupine and is particularly capable of the quantification of residues in sausage, bakery products, potato products, ketchup and juices. Due to high cross-reactivity the test is suitable for the detection of all commonly used lupine species.

PRINCIPLE OF THE TEST

The **DAI Lupine** quantitative test is based on the principle of the enzyme linked immunosorbent assay. An antibody directed against lupine proteins is bound on the surface of a microtiter plate. Lupine containing samples or standards are given into 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 lupine proteins 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 colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of lupine is directly proportional to the colour intensity of the test sample.

PRECAUTIONS

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

HEALTH AND SAFETY INSTRUCTIONS

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

REAGENTS

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are printed on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-lupine antibodies.
2. Lupine Standards (0; 2; 5; 15; 30 ppm of lupine): 5 vials with 1.0 mL each, dyed red, ready-to-use.
3. Conjugate (anti-lupin-peroxidase): 15 mL, dyed red, ready-to-use.
4. Substrate Solution (TMB): 15 mL, ready-to-use.
5. Stop Solution (0.5 M H₂SO₄): 15 mL, ready-to-use.
6. Extraction and sample dilution buffer (Tris): 2 x 120 mL as 10x concentrate, dyed red. Dilute 1+9 with distilled water. Stored at 4°C the diluted buffer is stable for at least one week. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
7. Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. Stored at 4°C the diluted buffer is stable for at least 4 weeks. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8. Plastic bag to store unused microtiter strips.
9. Instruction Manual.

Additional Instrumentation and Reagents (not provided)

Instrumentation

- 100 - 1000 µL micropipets
- Volumetric flask
- Analytical balance
- Mortar, mixer
- Water bath
- Centrifuge
- ELISA reader (450 nm)

Reagents

- double distilled water

SAMPLE PREPARATION

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. Lupine proteins could adhere to different surfaces. 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 solid 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 and sample dilution 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 2000 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. Due to high matrix effects samples containing more than 50% pure beef should be further diluted 1 + 1 with pre-diluted extraction and sample dilution buffer.
5. 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.

The following sample preparation should be applied for liquid samples:

1 mL of liquid sample is diluted in 19 mL of pre-diluted extraction and sample dilution 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. The process is continued at point 3 of solid sample extraction process.

PROCEDURE

The washing solution is supplied as 10x concentrate and has to be diluted 1+9 with double distilled water before use.

In any case the ready-to-use standards provided should be determined twofold. When samples in great quantities are determined, the standards should be pipetted once before the samples and once after the samples. For final interpretation the arithmetic mean is used for calculation.

In consideration of GLP and quality control requirements a duplicate measurement of samples is recommended.

The procedure is according to the following scheme:

1. Prepare samples as described above.
2. Pipet 100 µL ready-to-use standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.
3. Incubate for 20 minutes at room temperature.
4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
5. Pipet 100 µL of conjugate (anti-lupin-peroxidase) into each well.
6. Incubate for 20 minutes at room temperature.

7. Wash the plate as outlined in 4.
8. Pipet 100 μ L of substrate solution into each well.
9. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
10. Stop enzyme reaction by adding 100 μ L of stop solution (0.5 M H₂SO₄) into each well. The blue colour will turn yellow upon addition.
11. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

CALCULATION OF RESULTS

The ready-to-use standards are prepared for a direct determination of sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. Additional dilution due to high sample concentration has to be accounted for.

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ppm on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.
3. Using the mean optical density value for each sample, determine the corresponding concentration of lupin in ppm from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

TYPICAL STANDARD VALUES

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 30 ppm standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Lupine (PPM)	% binding of 30 ppm
30	100
15	75
5	38
2	21
0	5

PERFORMANCE

Sensitivity

The limit of detection (LOD) of the DAI Lupin test is 0.2 ppm for the standard curve. Validation experiments with common matrices resulted in the following LODs [ppm].

Sausage	0.2
Bread	0.3
Orange juice	0.7
Ketchup	0.1
Croquette	0.2

The limit of quantification (LOQ) of the DAI Lupin 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.

Precision

Intra-assay Precision	6-9%
Inter-assay Precision	5-9%

Linearity

The serial dilution of spiked samples (sausage, bread, orange juice, ketchup and croquette) resulted in a dilution linearity of 78% - 126%.

Cross-reactivity

For the following foods no cross-reactivity could be detected:

Milk	Sunflower seeds	Cocoa
Egg	Pumpkin seeds	Potato
Wheat	Cashew	Leek
Rye	Peanut	Pea
Oats	Hazelnut	Bean
Barley	Almond	Tomato
Rice	Pecan	Peach
Corn	Coconut	Cherry
Buckwheat	Brazil nut	Orange
Sesame	Walnut	Strawberry
Pork	Pistachio	Plum
Beef	Macadamia nut	Locast bean gum
Chicken	Chestnut	Guar gum

The following cross reactions were determined:

Chickpea	0.0003%
Lentil	0.0004%
Soy flour, unroasted	0.07%
Soy flour, roasted	0.0009%

Recovery

Mean recovery was determined by spiking samples with different amounts of lupin:

Sausage	99%
Bread	113%
Orange juice	104%
Ketchup	98%
Croquette	111%

REFERENCE

1. Rodriguez J, et al. (2010) - Characterization of lupin major allergens (Lupinus albus L.). Mol Nutr Food Res, published online
2. Rojas-Hijazo B, et al. (2006) - Unsuspected lupin allergens hidden in food. Int Arch Allergy Immunol, 141(1):47-50
3. Leduc V, et al. (2002) - Allergenicity of lupin flour. Allerg Immunol (Paris), 34(6):213-7
4. Dooper MM, et al. (2007) - Monoclonal antibodies against the candidate lupin allergens alpha-conglutin and beta-conglutin. Int Arch Allergy Immunol, 143(1):49-58
5. Prieto A, et al. (2010) - Recurrent anaphylaxis due to lupin flour: primary sensitization through inhalation. J Investig Allergol Clin Immunol, 20(1):76-9

Date Adopted	Reference No.
2010-06-25	DA-Lupin-2010



DIAGNOSTIC AUTOMATION, INC.
23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302
Tel: (818) 591-3030 Fax: (818) 591-8383

ISO 13485-2003



Revision Date: 12-6-2013