Version 20-04/24

User's Manual

Lupin ELISA Kit

REF **DEIA294**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

A Monoclonal Antibody-Based Enzyme-Linked Immunosorbent Assay (ELISA) for the Quantitative and/or Qualitative Detection of Lupin in Food

General Description

The Lupin ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for the quantitative or qualitative detection of lupin. This monoclonal antibody (MAb)-based assay provides a highly sensitive and specific method for the quantification of lupin presence/contamination within a variety of food matrices. It may also be used for simple "yes-no" qualitative assessments. The kit enables a facile and quantitative measure of lupin without the cross-reactivity issues often associated with polyclonal antibody (PAb)-based ELISA kits. The target indicator protein was selected based on its strong resistance to food processing and high abundance in a variety of commercial lupin flours.

Reagents And Materials Provided

Reagent	Amount within 48-well kit	Amount within 96-well kit
Assay plate, one 96-well plate	Six 8-well strips *Sufficient for 48 or 96 assay values, including standards & blank*	Twelve 8-well strips
10X ExB = 10X extraction buffer	50 mL *Sufficient to extract > fifty 0.5 g or 0.5 mL samples*	
5X SD = 5X sample diluent	50 mL *Sufficient to dilute samples for > 96 assays wells*	
Ready-to-use lupin calibrators: 40, 20, 10, 4, 1 and 0 ppm	1 mL of each standard *Sufficient for 5 standard curves*	2 mL of each standard *Sufficient for 10 standard curves*
10X WB = 10X wash buffer	50 mL *Sufficient wash buffer for > 96 wells*	
CON = 1X anti-lupin HRP-conjugate	12 mL *Sufficient for > 48 wells*	15 mL *Sufficient for > 96 wells*
SUB = high sensitivity TMB substrate	12 mL *Sufficient for > 48 wells*	15 mL *Sufficient for > 96 wells*
STOP = HRP quench solution	12 mL *Sufficient for > 48 wells*	15 mL *Sufficient for > 96 wells*

Materials Required But Not Supplied

1. Chamber or waterbath for 60°C incubation
2. Timer
3. Balance or scale capable of measuring milligram quantities
4. 1.5 or 2.0 mL microfuge tubes and tube racks
5. 15 or 50 mL conical tubes
6. Distilled water or equivalent
7. Pipet-Aid (or equivalent) and serological pipettes, capable of measuring 5-50 mL
8. *Single and multichannel pipettes, capable of measuring 1-1,000 µL

9. *96-well assay blocks
10. *Reagent reservoirs
11. Absorbent paper or ELISA plate washer
12. Centrifuge, capable of 2,000 x g
13. Vortex
14. Microplate reader, capable of reading absorbance at 450 nm

*Note- It is recommended that assay blocks, reagent reservoirs (boats) and a multichannel pipette be used in order to obtain the most accurate results. For assays in which more than 16 samples will be tested, this is required in order to insure the incubation time of all samples is universal.

Storage

Each plate is packed in a vacuum-sealed Mylar™ pouch with desiccant and oxygen absorbing packets to extend the shelf life of the product to a minimum of six (6) months from the date of manufacture, if stored at 4°C. The stability of the ready-to-use standards may slightly deteriorate over time, as indicated in the certificate of analysis accompanying each kit.

Specimen Collection And Preparation

To ensure adequate sampling, it is important that the samples be thoroughly homogenized and the particle size rendered as small as possible. Blending/grinding to a fine powder/flour is strongly recommended. Small particle size also enhances extraction efficiency.

Solid/Liquid sample extraction (read 'important notes' section prior to this step)

1. Transfer 0.5 gram of finely ground food matrix or 0.5 ml of liquid sample to a > 15 ml tube.
2. Add 9.5 ml of preheated 1X extraction buffer (diluted **ExB**) to mixture and briefly vortex to suspend the contents. If other starting quantities are used, a 20:1 buffer/sample ratio should be maintained.
3. Incubate tubes at 60°C (140°F) for 10 minutes, mixing vigorously every ~2 minutes.
4. Spin extraction samples at 2,000 x g for 10 minutes at room temperature and transfer the aqueous phase into a fresh tube for testing.

Swab sample extraction

1. Collection of environmental swab samples from surfaces should be carried out according to the manufacturer's protocol. If using the CD Swab Sample Kit, simply follow the provided sample collection instructions and proceed with the protocol below.
2. Add 4 ml of preheated 1X extraction buffer (diluted **ExB**) to the swab sample collection tube containing the 1 ml swab sample solution and detached swab head. Briefly vortex to mix the contents
3. Incubate tubes at 60°C (140°F) for 10 minutes, mixing vigorously every ~2 minutes.
4. The sample is now ready for testing using the recommended procedure below.

Reagent Preparation

Determine the amounts of reagents needed and dilute the concentrated extraction buffer (**10X ExB**), sample diluent (**5X SD**), and wash buffer (**10X WB**) using distilled water or equivalent.

• To prepare 100 ml of 1X ExB, add 10 ml of the **10X ExB** to 90 ml of distilled water. Prior to performing sample extraction, preheat the 1X ExB to 60°C (140°F).

• To prepare 100 ml of 1X SD, add 20 ml of the **5X SD** to 80 ml of distilled water.

• To prepare 100 ml of 1X WB, add 10 ml of the **10X WB** to 90 ml of distilled water.

Important notes

Extraction

The Lupin ELISA assay is extremely sensitive, capable of detecting minute amounts of lupin. Careful consideration should be given for the preparation of food matrices to ensure several important parameters:

1. The equipment used to prepare samples must be thoroughly cleaned to prevent the contamination of subsequent samples.
2. Disposable plasticware (tubes, pipette tips, etc.) are used wherever possible.
3. The samples are homogenized completely to prevent excessive intra-sample variation.
4. The supplied extraction buffer is sufficient for up to fifty 0.5-gram or 0.5-milliliter samples. If other quantities are used, a 20:1 buffer/sample ratio should be maintained (19 volumes of extraction buffer to 1 part sample).
5. Food matrices containing high concentrations of solid fats, such as chocolate, may require additional heating to completely melt the sample before adding preheated extraction buffer.
6. For spices, seasoning mixes, dairy products or food matrices containing polyphenols, such as tannins,found in chocolate, fruits, wine, tea and coffee, the addition of 5% non-fat dry milk to the extraction buffer is recommended to achieve optimal results. If performance issues with a certain matrix are suspected, please contact a CD representative.

Spike control preparation (optional)

Some food matrices may alter the recovery and sensitivity of the ELISA. If suspected, unspiked and lupinspiked control matrices can be tested. Values obtained on test samples can then be adjusted accordingly.

Ready-to-use standards provided with kit are meant to serve as calibrators for the assay and are NOT to be used as spiking agents.

Assay Procedure

It is important for the user to read all instructions carefully before performing the assay. Reagents, including the assay plate, should be equilibrated to room temperature prior to use.

1. Determine the number of assay wells needed for test samples and for standards. Carefully remove the strips that are not to be used by gently pushing them from beneath the plate until they pop out and return to Mylar bag. Seal and store at 2-8°C.
2. Add 200 µL of sample extract and ready-to-use standards to the appropriate wells.

***Note**- for unknown or known positive samples, testing additional dilutions of the extract can help ensure that a value within the assay ROQ is obtained. To prepare a 2-fold diluted sample, for example, mix 1 volume of extracted sample with 1 volume diluted 1X SD.

3. Incubate plate at room temperature for 10 minutes.
4. Discard well contents, blot onto absorbent paper with a slapping action (or autowash). Wash a total of 3X

with 1X wash buffer (diluted **WB**) using ≥ 200 µL per wash and blot dry.

5. Add 100 µL of 1X anti-lupin antibody-conjugate (**CON**) to each well.
6. Place plate in dark environment and incubate at room temperature for 10 minutes.
7. Discard well contents, wash, and blot dry as described in step #5.
8. Add 100 µL of HRP substrate (**SUB**) per well.
9. Incubate plate in dark for 10 minutes.
10. Add 100 µL of quench solution (**STOP**) to each well and mix by gently pipetting so as to prevent bubbles that could interfere with absorbance readings.
11. Read the absorbance of the wells using a plate reader programmed with a primary absorbance filter of 450 nm and a differential filter of 630 nm. For some plate readers, the differential filter may be automatically accounted for and reading only at 450 nm will be required. Please consult your reader manual for more information.
12. Plot the standard curve. Interpolate unknown data using the standard curve and appropriate dilution factor if applicable. If the sample extract is run undiluted, a dilution factor of 1 would be used. Background may be subtracted to normalize data if desired.

Quality Control

The ready-to-use standards used in the assay should yield OD values in line with those indicated on the accompanying lot-specific certificate of analysis. Significant deterioration in signal may indicate expiration of the reagents. If quantification is required and the OD of the test sample is above that of the 40 ppm lupin standard, further dilution of the sample should be performed prior to repeating the assay to ensure results fall within the assays ROQ.

Interpretation Of Results

A qualitative assessment can be made using one or more of the provided assay standards. Any of these standards can be used to define a specific threshold at which the unknown sample can be compared.Samples with normalized absorbance values above the threshold are determined to be positive, whereas those samples below the threshold are determined to be negative.

For swab samples, quantification of the target is generally not performed as the assay, is instead, intended to provide a qualitative indication as to whether or not lupin is present or absent in the test sample. The

CD Surface Swabbing Kit has been validated to recover at least 1 microgram lupin from a 25 cm^2 area using the swab method.

Quantitative analysis of assay results

A standard curve should be generated from the averaged ODs of the 0-40 ppm standards after subtracting the 0 ppm averaged background values. A third-order polynomial (cubic) curve fit is recommended for this evaluation. The ppm concentration of test samples can be determined by plotting OD values onto the curve and multiplying the calculated concentration by the appropriate dilution factor (if used). Note that the ppm designations on the provided standards are intended to allow the direct calculated ppm of total lupin in an original food sample. CD also offers users a MonoTrace ELISA calculation template in excel format which can be used to simplify sample quantitation. Please contact a representative for more information.

Detection Range

1 to 40 ppm

Detection Limit

0.13 ppm

Note- calculations of parameters are based on representative data from multiple assays using 10-minute incubation steps at room temperature (20-23.5°C / 68-74.3°F). Higher temperatures may result in elevated absorbance readings for samples and standards.

******The limit of detection (LOD) was determined statistically based on the standard deviation of the response (SD) and assay background according to the formula: LOD = background + 3X SD.

*******The range of quantitation (ROQ) was determined experimentally, whereby the lower limit of the ROQ is defined as the lowest concentration at which the assay can reliably and accurately quantify lupin in a sample. For quantitation above 40 ppm, samples should be diluted such that the results fall within the ROQ (1 to 40 ppm).

Specificity

The assay exhibits strong reactivity to lupin (Lupinus angustifolius, Lupinus albus, Lupinus luteus). At concentrations as high as 1,000,000 ppm, no crossreactivity was observed in a large panel of matrices including legumes, tree nuts, seeds, flour, meats, and spices.

Recovery

Recovery of spiked lupin samples was acceptable, according to current AOAC guidelines (J. AOAC Int. 2012), when the following food matrices were assayed: wheat flour, rice flour, oat flour, chickpea flour, lentil flour, chocolate, orange juice, wine, ketchup, crackers, fruit & nut trail mix, assorted baby foods, raw beef & sausage, red onion, steak & taco seasoning, soup mix, breads/cakes/cookies & associated mixes, and ice cream.

Assay Claims

When performed as instructed, the assay is capable of a simple yes/no qualitative assessment of Lupin presence in food samples or a quantitative determination of lupin content. Extracted food samples that generate a colorimetric readout can be compared to the linear portion of a standard curve, allowing the interpolation of lupin content in ppm. The assay is capable of quantifying lupin content between 1 and 40ppm.

A negative result by this or any other immunological assay does not assure the complete absence of lupin within the sample. The sample may contain lupin below the limit of detection of this kit. The Lupin ELISA kit does not claim that food is safe for consumption based upon a determination of lupin content.