3M

Product Instructions

# Pine Nut Protein ELISA Kit

**Enzyme-Linked Immunosorbent Assay (ELISA) for quantitative analysis of pine nut proteins.**

## Product Description and Intended Use

The 3M™ Pine Nut Protein ELISA Kit is intended for the detection of pine nut proteins in clean-in-place water (CIP) final rinse water, environmental swab samples, food ingredients, and processed food products.

The 3M Pine Nut Protein ELISA Kit utilizes a sandwich ELISA. The pine nut proteins present in the sample react with the anti-pine nut antibody, which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-pine nut antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound pine nut protein. Following a second washing step, the enzyme bound to the immunosorbent is detected by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The color development from this enzymatic reaction varies directly with the concentration of pine nut protein in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of pine nut protein in the test sample. The quantity of pine nut protein in the test sample can be extrapolated from the standard curve, constructed from standards of known concentration, and adjusted to consider the sample dilution.

The 3M Pine Nut Protein ELISA Kit is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than food or beverage. For example, 3M has not documented this product for testing pharmaceutical, cosmetic, clinical or veterinary samples. The 3M Pine Nut Protein ELISA Kit has not been evaluated with all possible food products, food processes and testing protocols.

The 3M Pine Nut Protein ELISA Kit contains 96 wells, described in Table 1.

**Table 1.** Kit components

| Item | Identification | Preparation (see Reagent Preparation section for details) | Storage | Stability |
|---|---|---|---|---|
| 3M™ Pine Nut Protein ELISA Wells  | One foil bag with a plate of 96 removable antibody coated wells. | Ready to use. | 2-8°C in sealed foil bag with desiccant. | Re-seal foil bag containing unused wells and desiccant. Store at 2-8°C to maintain stability until the expiration date of the kit. |
| 3M™ Pine Nut HRP Conjugate (10X)  | One vial with 1.5 mL of 10X Horseradish Peroxidase (HRP) Conjugated antibody (10X). | Dilute 1/10 immediately prior to use to make a 1X working solution. | 2-8°C in the dark. | The 10X conjugate is stable until the expiration date of the kit. |
| 3M™ Pine Nut Protein Standard Concentrate  | One vial with a known concentration of pine nut protein. | Refer to the ELISA Procedure Section for standard preparation. | 2-8°C. Do not freeze. | 3M Pine Nut Protein Standard Concentrate is stable until the expiration date of the kit. |
| 3M™ Diluent (5X)  | One bottle with 50 mL of 5X Diluent. | Dilute 1/5 immediately prior to use to make a 1X working solution. | 2-8°C | The 5X 3M Diluent Solution is stable until the expiration date of the kit. |

| | | | | |
|---|---|---|---|---|
| 3M™ Wash Solution (20X)  | One bottle with 50 mL of 20X wash solution. | Dilute 1/20 to make a 1X working solution. | 2-8°C for both 1X working solution and 20X Wash Solution concentrate. | The 20X 3M Wash Solution is stable until the expiration date of the kit. The 1X Wash Solution is stable for at least one week after preparation. |
| 3M™ Extraction Buffer E26 (4X)  | One bottle with 120 mL of 4X extraction buffer. | Dilute 1/4 to make a 1X working solution. The working solution should be heated to 50-60°C before use. | 2-8°C for both 1X working solution and 4X 3M Extraction Buffer concentrate. | The 1X Extraction Buffer and 4X 3M Extraction Buffer are stable until the expiration date of the kit. |
| 3M™ Chromogenic Substrate Solution  | One bottle with 12 mL of 3,3',5,5'-tetramethylbenzidine (TMB). | Ready to use. | 2-8°C in the dark. | Protect from light. The 3M Chromogenic Substrate Solution is stable until the expiration date of the kit. |
| 3M™ Stop Solution  | One bottle of 12 mL of 0.3 M sulfuric acid. | Ready to use. | 2-8°C | The 3M Stop Solution is stable until the expiration date of the kit. |

Materials not provided in the kit:

- Precision pipettes and pipette tips to collect 10 to 100 µL
- Test tubes
- Microtiter plate washer/aspirator
- Distilled or deionized water
- Microtiter plate reader
- Assorted labware for the preparation of reagents and buffer solutions
- Timer
- Vortex
- Shaking water bath or shaking incubator
- Orbital shaker

## Safety

The user should read, understand, and follow all safety information in the instructions for the 3M Pine Nut Protein ELISA Kit. Retain the safety instructions for future reference.

⚠ **WARNING:** Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.

**NOTICE:** Indicates a potentially hazardous situation, which, if not avoided, could result in property damage.

### ⚠ WARNING

**To reduce the risks associated with exposure to chemicals:**

- Dispose according to current local/regional/national/industry standards and regulations.
- The user must train its personnel in current proper testing techniques; for example, Good Laboratory Practices[1] or ISO/IEC 17025[2].
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents.
- Avoid skin contact with the 3M Stop Solution, see Safety Data Sheet for additional safety information.

**To reduce the risks associated with false-negative results leading to the release of contaminated product:**

- Store the 3M Pine Nut Protein ELISA Kit as indicated on the package and in the product instructions.
- Use the 3M Pine Nut Protein ELISA Kit for food and environmental samples that have been validated internally or by a third party.
- Follow the protocol and perform the tests exactly as stated in the product instructions.
- 3M has not documented the use of the 3M Pine Nut Protein ELISA Kit in industries other than food or beverage. For example, 3M has not documented this product for testing pharmaceutical, cosmetic, clinical or veterinary samples.

**To reduce the risks associated with inaccurate results leading to the release of contaminated product:**

- Always use the 3M Pine Nut Protein ELISA Kit by the expiration date.
- Always prepare working solutions using the 3M Pine Nut Protein ELISA Kit concentrated reagents at 20-25°C temperature.
- Do not freeze the 3M Pine Nut Protein Standard Concentrate.
- If Chromogenic Substrate Solution turns blue, do not use. Follow Good Laboratory Practices[1] to avoid cross-contamination of 3M Chromogenic Substrate Solution.

**NOTICE**

**To reduce the risks associated with inaccurate results:**

- Sample stability after extractions has not been evaluated. The ELISA procedure should be carried out, right after sample extraction.
- Handle 3M Pine Nut Protein Standards following Good Laboratory Practices[1] to prevent cross-contamination of samples.

Consult the Safety Data Sheet for additional information.

For information on documentation of product performance, visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

## User Responsibility

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at **www.3M.com/foodsafety**, or contact your local 3M representative or distributor for more information.

**As with all test methods used for food analysis the test matrix can influence the results.** When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may influence results. The food sample itself may influence results.

It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of any 3M Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

## Limitation of Warranties/Limited Remedy

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods Authorization.

## Limitation of 3M Liability

3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase price of the product alleged to be defective.

## Storage and Disposal

Store 3M Pine Nut Protein ELISA Kit contents at 2-8°C. Do not freeze. Store diluted working solutions as described in Table 1.

3M Pine Nut Protein ELISA Kit components should not be used past the expiration date. Expiration date and lot number are noted on the outside label of the box.

Dispose according to current local/regional/national/industry standards and regulations.

## Instructions for Use

Follow all instructions carefully. Failure to do so may lead to inaccurate results.

## Reagent Preparation

Bring all reagents to ambient temperature (20-25°C) before use. Use clean labware to dilute and store working solutions.

**a. 3M Extraction Buffer**

To prepare 1X Extraction Buffer, add one part of 3M Extraction Buffer (4X) and dilute in three parts of deionized or distilled water. Pre-warm the Extraction Buffer (1X) to 50-60°C in a water bath or shaking incubator before use. Each sample requires 4.5 mL of 1X Extraction Buffer.

**b. 3M Diluent Solution**

To prepare 1X Diluent solution, add one part of 3M Diluent (5X) to four parts of deionized or distilled water. Each sample requires a total of 4.5 mL of 1X Diluent solution.

**c. 3M Wash Solution**

To prepare 1X Wash Solution, add one part of 3M Wash Solution (20X) to 19 parts of deionized or distilled water. Each 3M ELISA Well requires approximately 2.5 mL of 1X Wash Solution.

Note: The formation of crystals in the 3M Wash Solution (20X) may occur when stored at 2-8°C. To dissolve crystals, warm the 3M Wash Solution (20X) to 30-35°C in a water bath or incubator before preparing the Wash Solution (1X).

**d. 3M Pine Nut HRP Conjugate**

To prepare 1X Pine Nut HRP Conjugate, add one part of 3M Pine Nut HRP Conjugate (10X) and dilute in 9 parts of **1X Diluent solution**. Prepare immediately before use. Each 3M ELISA Well requires 100 µL of 1X Pine Nut HRP Conjugate.

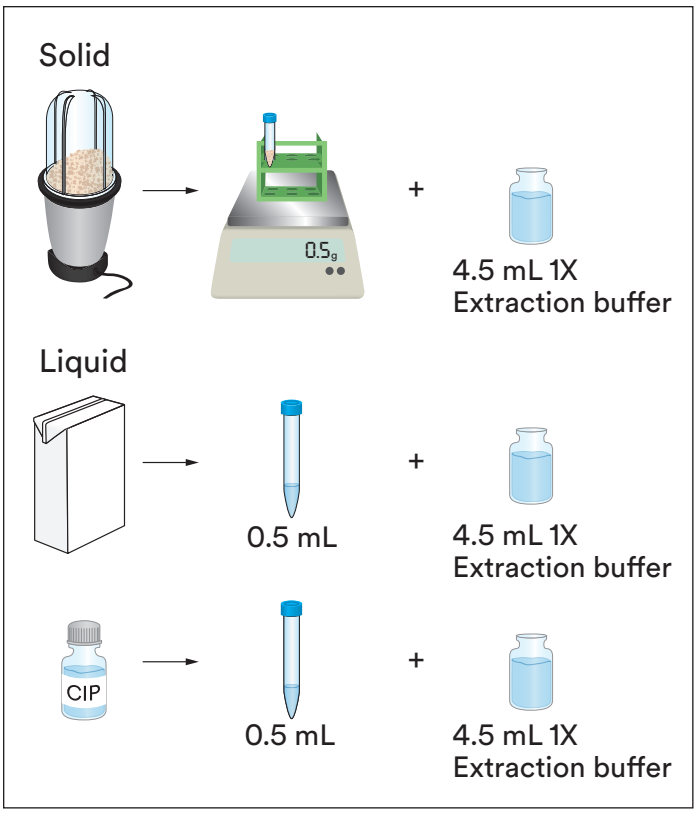
## Sample Preparation

Note: All samples should be extracted with 1X Extraction Buffer pre-warmed to 50-60°C.

1.1 Prepare sample for protein extraction in a clean test tube or disposable tube as described in Table 2.

**Table 2**. Sample preparation

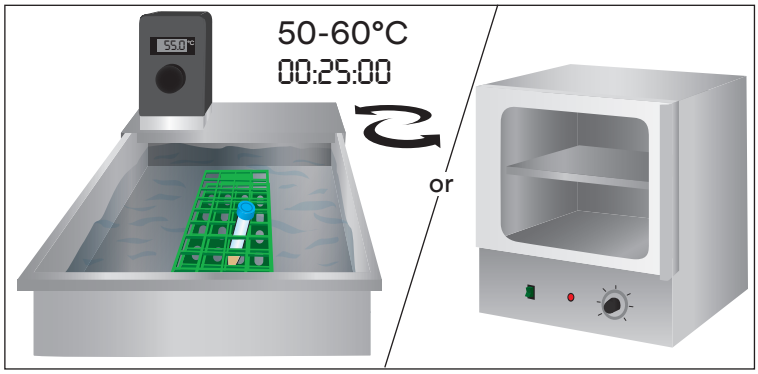
| Sample matrix | Sample size | Dilution (1/10) |
|---|---|---|
| Solid foods | 0.5 ± 0.02 g | Add 4.5 ± 0.09 mL of pre-warmed 1X Extraction Buffer |
| Liquid foods | 0.5 ± 0.01 mL | Add 4.5 ± 0.09 mL of pre-warmed 1X Extraction Buffer |
| Clean-in-Place (CIP) Final Rinse Water | 0.5 ± 0.01 mL | Add 4.5 ± 0.09 mL of pre-warmed 1X Extraction Buffer |

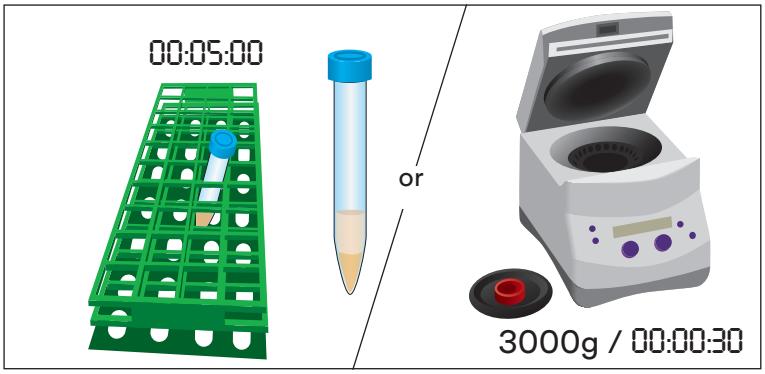


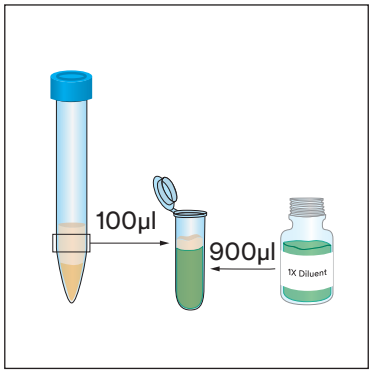

1.2 Incubate diluted samples in a shaking water bath or shaking incubator at 50-60°C for 25 ± 1 minutes. Another option is to leave the samples in a water bath or incubator at 50-60°C and manually shake for 1 minute every 5 minutes.

1.3 After incubation, centrifuge samples at 5000-7000 rpm (3000 x *g*) for 20 to 30 seconds to pellet particulates or allow them to settle for 5 minutes in a test tube rack.

1.4 Collect a 100 µL from middle (aqueous) layer and add it to 900 µL of Diluent Solution (1X). Vortex or shake to mix well. (This corresponds to a 1/100 dilution of the original sample.)



## ELISA Procedure

2.1 Remove one 3M ELISA Well per sample and/or standard and place the wells in the well holder. Return the unused 3M ELISA Wells to the foil pouch, re-seal and return to storage at 2-8°C.

2.2 Utilizing the 3M Pine Nut Protein Standard Concentrate, prepare a set of five standards diluted in Diluent Solution (1X).

| Standard Number | Standard Concentration (ng/mL) | Volume of standard added to 1X Diluent | Volume of 1X Diluent solution |
|---|---|---|---|
| 5 | 405 | 10 µL of 3M Pine Nut Protein Standard Concentrate | 990 µL |
| 4 | 135 | 200 µL of standard number 5 | 400 µL |
| 3 | 45 | 200 µL of standard number 4 | 400 µL |
| 2 | 15 | 200 µL of standard number 3 | 400 µL |
| 1 | 5 | 200 µL of standard number 2 | 400 µL |
| 0 | 0 | 0 | 400 µL |

2.3 Pipette 100 µL of each standard into 3M ELISA Wells.

- Standard 0 (1X Diluent Solution)
- Standard 1 (5 ng/mL) ppb
- Standard 2 (15 ng/mL) ppb
- Standard 3 (45 ng/mL) ppb
- Standard 4 (135 ng/mL) ppb
- Standard 5 (405 ng/mL) ppb

2.4 Pipette 100 µL of the extracted sample prepared in 1.4 into a 3M ELISA Well.

2.5 Incubate 3M ELISA Wells on an orbital shaker set at 400 rpm at ambient temperature (20-25°C) for 30 ± 2 minutes. Keep the wells covered and level during this step to prevent evaporation.

2.6 After incubation, aspirate the contents of the 3M ELISA Wells.

2.7 Fill completely each 3M ELISA Well with 1X Wash Solution and aspirate. If the wash is done manually, invert the plate and pour/shake out the contents in a waste container and strike the wells sharply on absorbent paper to remove residual wash solution. Repeat this step three times for a total of four washes.

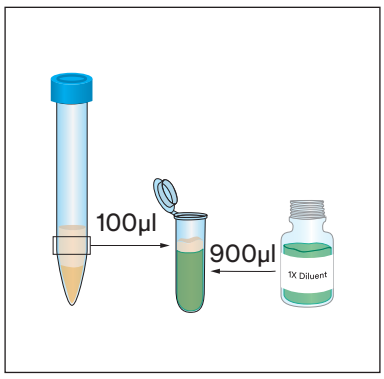
2.8 Pipette 100 µL of 1X Pine Nut HRP Conjugate into each 3M ELISA Well. Incubate on an orbital shaker set at 400 rpm at ambient temperature for 10 ± 2 minutes. Keep plate covered in the dark and level during this step.

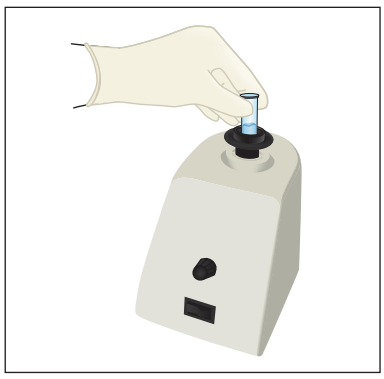
2.9 Repeat steps 2.6 and 2.7 to complete a total of four washes with Wash Solution (1X).

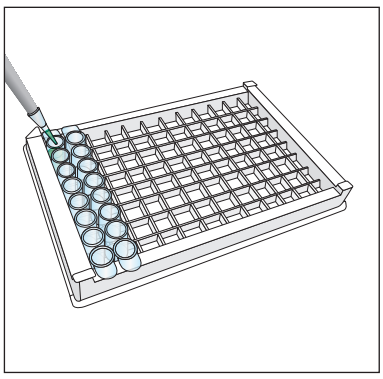
2.10 Pipette 100 µL of 3M Chromogenic Substrate Solution (TMB) into each 3M ELISA Well.

2.11 Incubate on an orbital shaker set at 400 rpm at ambient temperature for 10 minutes. Keep plate covered in the dark and level during this step.

2.12 After incubation, add 100 µL of 3M Stop Solution to each 3M ELISA Well and determine the absorbance (at 450 nm) within 30 minutes.








## Result Analysis

3.1 Subtract the average background value for each sample (Average absorbance reading of the sample minus average absorbance reading of standard zero.)

3.2 Using a computer software capable of generating a four parameter logistic curve fit, construct a standard curve by plotting the concentration in ng/mL (ppb) on the x-axis and the absorbance reading to each corresponding standard on the y-axis. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.

3.3 Calculate the sample concentrations off the standard curve; the result unit is in ng/mL (ppb). Then, multiply by sample dilution factor to get the concentration of original sample. For example, if the total dilution of the sample is 1/100, and the sample concentration of the standard curve is 200 ng/mL (ppb), the final sample concentration is 200 ng/mL x 100 = 20,000 ng/mL (ppb) which is 20 µg/mL (ppm).

## Minimum Performance Characteristics

a. The analytical Limit of Detection (LOD) is 1.3 ng/mL (ppb)

The limit of detection is defined as the lowest concentration of the allergen in a test sample that can be distinguished from a true blank sample at a specified probability level[3]. It is determined by adding three standard deviations to the mean optical density value of forty-eight standard zero replicates and calculating the corresponding concentration.

b. The Limit of Quantification (LOQ) is 0.5 ppm

The limit of quantification is defined as the lowest level of the allergen in a test sample that can be reasonably quantified at a specified level of precision[3].

## Precision

| | | |
|---|---|---|
| Intra-Assay Precision | Average %CV = <10% | N=12 |
| Inter-Assay Precision | Average %CV = <10% | N=12 |

## Specificity and Cross-Reactivity

This assay recognizes pine nut protein and was tested versus various samples for cross-reactivity (Table 3.)

**Table 3**. Cross-reactivity of the 3M Pine Nut Protein ELISA Kit.

| Matrix sample | % Cross-reactivity |
|---|---|
| Almond flour | <1% |
| Almond Milk | <1% |
| BLG | <1% |
| Bovine Casein | <1% |
| Bovine Milk | <1% |
| Brazil Nut | <1% |
| Buckwheat flour | <1% |
| Cashew | <1% |
| Celery | <1% |
| Chickpea | <1% |
| Coconut flour | <1% |
| Coconut Milk | <1% |
| Corn flour | <1% |
| Fish Parvalbumin | <1% |
| Hazelnut | <1% |
| Lima Bean | <1% |
| Macadamia Nut | <1% |
| Mustard Seed | <1% |
| Ovomucoid | <1% |
| Pea Extract | <1% |
| Peanut flour | <1% |
| Pecan | <1% |
| Pine Nut | (+) |
| Pistachio flour | <1% |
| Pumpkin Seed | <1% |
| Scallop | <1% |
| Sesame Seed | <1% |
| Shrimp | <1% |
| Sorghum flour | <1% |
| Soy flour | <1% |
| Soy Milk | <1% |
| Sunflower Seed | <1% |
| Walnut | <1% |

## References

1. U.S. Food and Drug Administration. Code of Federal Regulations, title 21, part 58. Good Laboratory Practices for Nonclinical Laboratory Studies.
2. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
3. Abbott, M., Hayward, S., Ross, W., Godefroy, S.B., Ulberth, F., Van Hengel, A. J., Roberts, J., Akiyama, H., Popping, B., Yeung, J.M., Wehling, P., Taylor, S., Poms, R.E., and Delahaut, P. (2010). Appendix M: Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices. *J. AOAC Int.* 93, 442-450.

## Explanation of Symbols

www.3M.com/foodsafety/symbols

## 3M Food Safety

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