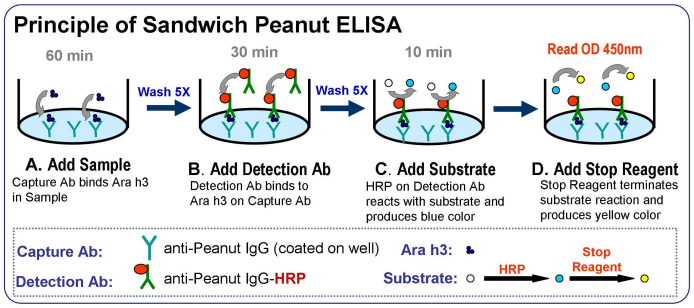
EnzyChrom™ Peanut ELISA (EPNT-100)

Colorimetric Sandwich ELISA for Peanut

DESCRIPTION

Peanut allergy is one of the most common and most dangerous food allergies. Trace amounts of peanut can be enough to trigger life-threatening anaphylaxis, which if left untreated, can result in death. Due to the severity and prevalence of peanut allergies, regulation of peanut containing foods is strictly enforced. The allergens Ara h1, Ara h2, Ara h3, and Ara h6 are the most prominent peanut allergens that cause allergic reactions. The protein Ara h3 is heat stable and comprises a significant portion of the protein in peanuts. These traits make Ara h3 an ideal candidate for quantifying peanut content in samples, even those that have been cooked or baked.

BioAssay Systems' Peanut ELISA uses a colorimetric sandwich ELISA method specific for the protein Ara h3 to quantify the peanut content of samples.



KEY FEATURES

Fast and Simple. Entire assay procedure takes less than three hours.

Sensitive and Accurate. Quantifies peanut in the parts per billion range.

Simple Calculation. Simple calculation with linear standard curve.

Detection range: (5 – 50 ppb).

APPLICATIONS

Determination of peanut in food and beverage samples.

KIT CONTENTS

10× Wash Buffer:	50 mL	**TMB Buffer:**	14 mL
10× Sample Buffer:	25 mL	**Stop Reagent:**	14 mL
TMB Substrate:	75 µL	**Detection Ab:**	75 µL
Standard (10,000 ppb):	50 µL	**Capture Stripwell Plate:**	1

Storage conditions: this kit is shipped on ice. Upon delivery, store Detection Ab and Standard at -20°C. Store all other reagents at 4°C. Shelf life of 12 months after receipt.

Note: all reagents may be stored at -20°C; however, precipitates may form in the 10× Wash Buffer and 10× Sample Buffer. If precipitates form, heat them in a hot water bath until the precipitates are solubilized before use.

Precautions: reagents are for research use only. Briefly centrifuge tubes before opening. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURE

Important:

1. To avoid cross-contamination, change pipette tips between additions of each reagent or sample. We recommend the use of a multi-channel pipette. Use separate reservoirs for each reagent. Prior to assay, dilute 10× Wash Buffer in dH_2O to prepare 500 mL 1× Wash Buffer. Dilute 10× Sample Buffer in dH_2O to prepare 250 mL 1× Sample Buffer.
2. It is recommended that samples be assayed in duplicate or higher.
3. Please see "Materials Required But Not Provided" section for a list of additional reagents and equipment that are needed.

A. Sample Preparation

1. Thoroughly homogenize sample to a fine consistency in a clean, contamination free homogenizer. Samples that are already a fine consistency (e.g. powders, pastes, liquids) can skip this step.
2. Weigh out 1 g of finely ground homogenized sample in a clean, disposable 15 mL polypropylene centrifuge tube. For liquid samples, add 1mL of sample to a clean, disposable 15 mL polypropylene centrifuge tube.
3. Add 9 mL 1× Sample Buffer and vortex 1 – 2 minutes to mix thoroughly, ensuring the sample is homogeneous. Add 20 µL 2-Mercaptoethanol. This makes a 10% w/v solution for solid samples and a 10% v/v solution for liquid samples.
5. Place tube in a 60°C waterbath and incubate for 10 minutes. Remove tube from heat source and rinse under cold water to cool to RT.
7. Mix and vortex tube thoroughly 1 – 2 minutes ensuring the sample is again completely homogeneous. Aliquot 1 mL to a 1.5 mL centrifuge tube. For thick, viscous solutions you may wish to widen the pipette by cutting off a portion of the tip. Centrifuge for 10 minutes at 5,000 g. Collect the supernatant as the Sample Extract. *Note: there may be a layer of fat collected on the top of the extract. Pipette around or discard this layer.*
8. Dilute the Sample Extract in 1× Sample Buffer. For unknown samples, it is advised to run a serial dilution to determine the best dilution factor to use (e.g. high peanut content samples such as peanuts and peanut butter need to be further diluted 50,000 – 5,000,000-fold). *Note: be sure to factor in that the Sample Extract is a 10% w/v or v/v solution when correcting for dilution factor in the calculation.*

B. Kit Reagents

1. Bring kit Reagents to RT. Remove capture stripwell plate from vacuum sealed bag and takeout required number of strips to accommodate all samples and standards to be run. Keep Detection Ab on ice or at 4°C when not in use. Return unused strips to bag and store at 4˚C.

C. Standards

1. Prepare 1 mL 50 ppb standard: mix 5 µL 10,000 ppb Peanut Standard with 995 µL 1× Sample Buffer.
2. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

Standard No.	[Standard]	Volume of Sample Buffer	Volume of 50 ppb Standard
1	50 ppb	0 µL	500 µL
2	30 ppb	200 µL	300 µL
3	15 ppb	350 µL	150 µL
4	0 ppb	500 µL	0 µL

D. Peanut Capture

1. Add 100 µL of Standards to wells in duplicate. Add 100 µL of appropriately diluted Samples to wells in desired number of replicates (e.g. duplicates, triplicates, quadruplicates, etc).
2. Incubate 1 hour at room temperature.
3. Aspirate wells and wash 5× with 300 µL 1× Wash Buffer.

E. Add Detection Antibody

1. For each 8-well strip prepare 1 mL of Detection Reagent as follows: 5 µL Detection Ab + 1 mL 1× Wash Buffer.
2. Add 110 µL Detection Reagent to each well.
3. Incubate 30 min at room temperature.
4. Aspirate wells and wash 5× with 300 µL 1× Wash Buffer.

E. Detection

1. For each 8-well strip prepare 1 mL TMB Reagent as follows: 5 µL TMB Substrate + 1 mL TMB Buffer.
2. Quickly add 100 µL TMB Reagent to each well.
3. Incubate plate 30 min protected from light.
4. Quickly add 100 µL Stop Reagent to each well.
5. Read OD at 450 nm.

CALCULATION

Subtract blank value (Standard #4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the peanut concentration of Sample as follows:

$$[\text{Peanut}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}} \times n \ \text{(ppb)}$$

where OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Blank (Standard #4), respectively. n is the sample dilution factor.

Note: if the sample OD value is higher than OD for the 50 ppb peanut standard, further dilute the sample in 1× Sample Buffer and repeat the assay.

Conversions:

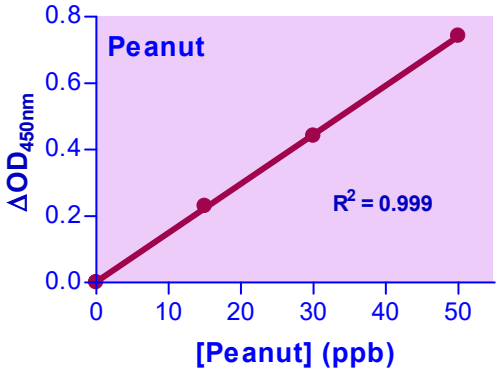
1 mg/L = 1 ppm = 1,000 ppb;

1 mg/mL = 1,000 ppm = 10^6 ppb;

1% = 1 g/dL = 10,000 ppm = 10^7 ppb

MATERIALS REQUIRED BUT NOT PROVIDED

2-Mercaptoethanol; 15 mL centrifuge tubes; deionized or distilled water; pipetting devices; 1.5 mL centrifuge tubes; table centrifuge; 60°C water bath; colorimetric plate reader capable of reading at OD 450 nm.



Peanut Standard Curve

LITERATURE

1. Hindley JP, et al (2018). Dose of allergens in a peanut snack (Bamba) associated with prevention of peanut allergy. J Allergy Clin Immunol. 141(2): 780-782.
2. Hourihane JO, et al (2017). Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge study to validate eliciting doses in children with peanut allergy. J Allergy Clin Immunol. 139(5):1583-1590.
3. Ramesh M, et al (2015). Peanut T-cell epitope discovery: Ara h1. J Allergy Clin Immunol. 137(6) :1764-1771.