Read instructions carefully before starting test

BioKits
Egg Assay Kit

Store at 2–8°C (35–46°F)

Sandwich enzyme immunoassay for the detection and quantification of egg white protein (EWP) in environmental swabs and food products by enzyme assay

SPECIFICATIONS

Limit of Detection:	0.1 ppm egg white protein (EWP) The limit of detection (LOD) was statistically determined by extrapolation of the allergen concentration at an OD value of zero (the average OD value for the zero replicates, over 10 separate assays) + 3 times the standard deviation of the OD results.
Range of Quantification:	0.5–10 ppm
Units of Measurement:	Egg white protein
Calibration:	NIST SRM8415 whole egg powder extract; no certified reference material available
No. of Determinations:	48 (including standards and controls)
Sample Preparation:	Buffer preparation, shaking and centrifugation
Time Required:	Sample extraction time: approx. 40 minutes (5 samples) Test incubation time: 75 minutes
Specificity:	The polyclonal antibody specifically detects Ovomucoid (*Gal d1*)
Cross-reactivity:	Of a large panel of commodities including nuts, pulses, grains, and proteins, none were found to be cross-reactive in the assay above the lower limit of quantification.

CALIBRATION

Units	Quantification
Egg White Protein	0.5–10 ppm
Ovomucoid (10% of EWP*)	0.05–1 ppm
Whole Egg Powder	1.5–30 ppm

Conversion based on the presence of whole egg powder.

*Internet Symposium on Food Allergens: www.food-allergens.de

SAFETY / COSHH NOTE

"Good Laboratory Practice" techniques should be employed when using this kit; if such practices are used the reagents constitute a very low potential risk to health. Safety clothing (lab coat, glasses and gloves if necessary) should be worn and skin contact with reagents avoided; do not ingest. Any contact with skin/eyes should be treated by washing/irrigation. It is also important to be aware of the allergic, toxic or infectious potential of analytical samples.

KIT COMPONENTS

Each kit contains sufficient material for 48 measurements (including standards and controls). The following components are provided in each kit:

Component	Detail	Vials / Bottle	Ready-to-Use
Egg Standard S1	0.5 ppm EWP	1	✔
Egg Standard S2	1 ppm EWP	1	✔
Egg Standard S3	2 ppm EWP	1	✔
Egg Standard S4	5 ppm EWP	1	✔
Egg Standard S5	10 ppm EWP	1	✔
Biscuit Crumb	<0.1 ppm EWP content	1	✔
Egg Spike Control	USE WITH CARE: Contains high levels of egg extract	1	✔
Anti-Ovomucoid Biotin		1	✔
Avidin Peroxidase Conjugate		1	✔
TMB Substrate		1	✔
Wash Solution Concentrate		1	10-fold concentrate
Diluent Concentrate Type 8		1	5-fold concentrate
Stop Solution	USE WITH CARE: STRONG ACID	1	✔
Anti-Ovomucoid Sensitized Microwell Module	48 Microwells (6 x 8 strips + frame)	N/A	✔
Package Insert and Blank Result Form			

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents (all Analytical or equivalent grade)

- Sodium chloride
- 1 M hydrochloric acid
- Tris (hydroxymethyl) methylamine
- Teleost gelatin (Sigma G7765)

Equipment

- Pestle and mortar, stomacher or suitable grinder/mill, which can be easily cleaned after use to eliminate the possibility of cross-contamination between samples
- Miscellaneous laboratory plastic and/or glassware, including measuring cylinders, pipettes, disposable Pasteur pipettes, plate seals and containers suitable for food extracts
- pH Meter
- Water bath capable of maintaining 60°C ± 2°C

- Wrist action or similar shaker
- Validated microwell plate shaker set at 700–800 rpm
- Centrifuge and appropriate centrifuge/microfuge tubes for clarifying sample extract
- Precision micropipette(s) capable of delivering 50 and 100 microliters, plus disposable tips
- Microwell washer (e.g. NUNC Immuno Wash 8) or wash bottle
- Microwell plate reader, fitted with 450 nm interference filter (calibrate regularly)

PREPARATION AND EXTRACTION OF SAMPLES

Note: The assay is extremely sensitive to the presence of egg material. As an indication, <0.5 milligrams of egg material can be detected in approximately one kilogram of food. **However, such a limit of detection assumes that food samples can be ADEQUATELY SAMPLED prior to extraction and also that they can be rendered truly HOMOGENEOUS.**

It is strongly recommended that due note is made of the order in which samples are processed so that the likelihood of a positive result that is actually a "false positive" (e.g. when a negative sample is processed immediately after a strongly contaminated one) can be assessed.

Because of the sensitivity of the method disposable tubes/containers should be used where possible and **great care must be taken** to thoroughly clean all reusable equipment, glassware, etc., between samples to avoid cross contamination.

PREPARATION OF SAMPLE EXTRACTION BUFFER

Note: The same sample extracts can also be used for the following assay kits: Almond, Hazelnut, Peanut and Sesame.

Prepare high salt Tris extraction buffer for the extraction of food samples. Amounts quoted are for 1.5 liters, the volume required for 28 samples (~55 mL per sample to be extracted).

1. Dissolve 9.1 g (± 0.1 g) Tris and 17.5 g (± 0.1 g) sodium chloride in ~1 L purified water.
2. Add 150 g gelatin (Sigma G7765) and stir well to dissolve.
3. Adjust pH to 8.15–8.25 with 1M hydrochloric acid; make up volume to 1,500 mL (± 10 mL).

ENZYME IMMUNOASSAY PREPARATION

1. Prepare diluted sample extracts, controls and kit materials.
2. Remove all reagents from the kit box and allow to reach ROOM TEMPERATURE (18–22°C; 64–72°F) before starting the test.
3. **Egg standards, egg spike control, anti-ovomucoid biotin, Avidin peroxidase conjugate, TMB substrate and stop solution** are supplied ready to use. NO PREPARATION is necessary, simply mix by repeated inversion (do not shake).
4. **Wash solution concentrate:** Supplied as a 10-fold concentrate. Dilute 1:9 in purified water to prepare working wash solution. For example, add 100 mL (± 1 mL) to a volumetric flask/cylinder and make up to 1.0 liter (± 10 mL) with purified water.
5. **Diluent concentrate type 8:** Supplied as a 5-fold concentrate. Dilute 1:4 in purified water to prepare working diluent solution. For 48 microwells, add 30 mL (± 0.5 mL) to a volumetric flask/cylinder, and make up to 150 mL (± 1.5 mL) with purified water. For any other number of microwells, dilute 1:4 with water, e.g. for a group of 24 microwells add 15 mL (± 0.2 mL) to 60 mL (± 1 mL) of purified water.

Note: The diluent concentrate may produce crystals after refrigerated storage. **These crystals should be redissolved before use.** Warming to room temperature, with occasional mixing, should dissolve the salt crystals. If warming to room temperature is not sufficient, then warming to 40°C with mixing will be required.

6. **Anti-ovomucoid sensitized microwell module:** Open the foil pouch. Take out the microwell module, remove the microwell strips not required and return them to the pouch taking care that the desiccant is present; reseal the pouch carefully.

   Note: With a pencil, number the columns in sequence on the upper frosted edge of the strips in use; this preserves the identity of the strips should they become detached from the frame.

PREPARATION AND EXTRACTION OF SAMPLES / CONTROLS

Sample the material to be tested; prepare by stomaching, grinding, blending or mincing. It is important to reduce the particle size of the material as far as possible, if possible to a flour or paste, while avoiding the potential for cross-contamination.

1. Weigh out at least 5 g of each sample or blank biscuit crumb control into a clean container. If appropriate (e.g. for chocolate) melt sample in water bath before adding hot extraction buffer.

Controls

Assay Control (recommended)

Spike the blank biscuit crumb with 20 microliters per gram (i.e. 100 µL for 5 g) of egg spike control. Mix well and set aside for at least 30 minutes while preparing other reagents and equipment. This will prepare a spiked assay control sample containing the equivalent of ~1.6 ppm SRM8415 egg white protein.

Liquid Control

If users do not wish to prepare a spike recovery assay control using biscuit crumb, a liquid control may be prepared by diluting 20 µL of egg spike control in 10 mL of extraction buffer. This dilution must be diluted further (1:9 in working diluent) prior to assay.

2. Pre-heat extraction buffer to 60°C in a water bath.
3. Add hot extraction buffer at a ratio of 10 mL (± 0.1 mL) per gram of sample into the container containing sample to be extracted.
4. Stomach samples for 120 seconds or mix for 15 minutes at room temperature.
5. Remove a portion of the extracted sample slurry with a disposable Pasteur pipette, place into an Eppendorf tube, seal and centrifuge at ~10,000 g for 10 minutes. Alternatively, allow the mixture to settle until a liquid extract layer appears.
6. With a clean disposable Pasteur pipette remove a portion of the aqueous extract from below any fat that might be present and above the settled layer; place into a second, clean tube/container. Mix/vortex well.
7. Dilute the settled/centrifuged sample or control extracts 1:9 in working diluent solution: add 100 µL (± 1µL) of extract to 900 µL (± 9 µL) of working diluent in a clean glass/plastic container. Mix/vortex well.
8. The diluted sample extract is now ready for testing.

ENVIRONMENTAL SWAB SAMPLE PREPARATION

For the preparation of environmental swab samples the Biokits Allergen Swabbing Kit (BASK) is required (Cat No. 901042J). This kit can be used in conjunction with the Egg Assay Kit for the determination of egg contamination levels in the environment.

TEST PROCEDURE

It is recommended that, for practice assays, small runs are performed and duplicate wells used for all samples. When good precision is being achieved (replicate OD450 nm %CVs < ~15%) reaction wells may be run singly. However, it is good laboratory practice that duplicates are run for some or all diluted extracts and imprecision (%CV of OD450 nm and concentration values) estimated in all assays as part of an ongoing QC program.

Note: When testing has been started, all steps should be completed without interruption to reduce the possibility of assay "drift" across the microwells.

1. Prepare diluted sample extracts and kit materials.
2. Add 100 µL (± 1 µL) of working diluent (used as zero standard), egg standard(s) S1–S5 and each of the diluted assay controls and sample extracts into appropriate microwells.

   Note: Use a separate disposable tip for pipetting each standard/diluted sample extract to prevent cross-contamination.
3. Incubate for 30 MINUTES at room temperature, static, or 15 minutes at room temperature, shaken.
4. Discard the liquid from the microwells, then (using NUNC Immuno Wash 8 or wash bottle) completely fill all wells with working wash solution. Discard the liquid and repeat the fill and discard sequence 4 more times (5 washes in total). Following the final discard, tap the plate upside down on several layers of absorbent tissue to completely remove residual droplets/bubbles of wash solution.
5. Add 50 µL (± 0.5 µL) of anti-ovomucoid biotin to all wells.
6. Mix the strips in the frame gently by hand on a flat surface and allow to incubate with vigorous SHAKING for 15 MINUTES at room temperature. (Alternatively allow to incubate STATIC for 30 MINUTES at room temperature).
7. At the end of the incubation, repeat the wash sequence used in Step 4.
8. Add 50 µL (± 0.5 µL) of Avidin peroxidase conjugate to all wells. Work from top to bottom of each strip as previously described.
9. Mix the strips in the frame gently by hand on a flat surface and allow to incubate for 15 MINUTES at room temperature (shaken or static).
10. At the end of the incubation, repeat the wash sequence described in Step 4.
11. Add 100 µL (± 1 µL) of TMB substrate to all wells.
12. Mix the strips gently by hand on a flat surface and incubate for 15 MINUTES at room temperature static.

    Note: The rate of color development is dependent on laboratory conditions and should be monitored in order to obtain suitable OD450 nm levels.
13. Add 50 µL (± 0.5 µL) of stop solution to all wells. Mix gently by hand to distribute the stop solution and prevent further color development. Color changes from blue to yellow and intensifies.
14. Using a microplate reader fitted with a 450 nm filter blank the reader on "Air" then measure and record the absorbance of each of the microwells.

    Note: Readings should be completed within 10 minutes of adding stop solution.

RESULTS

Note: it is assumed that ovomucoid comprises 10% of the dry weight of EWP. If whole egg powder is present in the sample, the limits of quantitation will equate to approximately 0.5–10 ppm egg white protein.

Qualitative

For qualitative assessment an individual egg standard can be used to define a specific (X ppm) cutoff level from the average OD450 nm.

Samples with absorbance values below the cutoff are classified as: NEGATIVE <X ppm

Samples at or above the cutoff are classified as: POSITIVE >X ppm

Quantitative

Quantitative estimates of EWP content can be obtained by using a calibration curve; to construct the calibration curve, use two-cycle log graph paper. Plot the mean absorbance value for each of the egg standards (0.5, 1, 2, 5 and 10 ppm) and fit a best curve to join each neighboring point. Alternatively, the results can be calculated using a graphical data reduction package using a suitable line fit for the curve. A linear regression (y=mX + C) can be used if only standards S1 to S4 are plotted.

Values returned when interpolating off the EWP assay curve line correspond approximately to EWP content in the ORIGINAL SAMPLE (assuming that the nominal extraction and dilution conditions are adhered to). **If additional dilutions are performed then the necessary factor needs to be applied to the recorded egg content.**

Report samples with an OD450 < standard S1 as "<LOQ". Report samples with an OD450 > standard S5 as ">10 ppm".

Poor replication (e.g. six zero standard wells %CV >12.5–15%) may indicate inadequate washing, contamination of the TMB substrate or splashing of Avidin peroxidase. Such imprecision is an indication of a problem during the performance of the assay, which may be INVALID and need to be repeated.

INTERPRETATION

The variability of raw material/product sampling, food composition (salinity, acidity, etc.) treatment of foodstuffs during processing (heat, pressure, etc.), difficulty of obtaining complete homogeneity during extraction and the reactivity of different sources of egg material means that the amount of detectable egg protein in the extract may vary considerably. Note: the NIST SRM8415 used to prepare the egg spike control has significantly lower activity (~30%) when compared to commercially available egg white / whole egg powders tested in-house.

Note: If a food sample gives a negative result in the test **it may still contain egg material** which is either unreactive in the test or below the limit of detection. **It should not be assumed that the food is "egg-free".**

ESTIMATION OF EGG PROTEIN CONTENT FROM SWAB SAMPLES

Approximate estimates of the quantity of EWP present in the swab solution are taken from the EWP calibration curve as follows:

A swab sample (no dilution) giving an absorbance which extrapolates to 2 ppm on the EWP standard curve contains 20 ng/mL EWP. This factor of x10 and conversion to ng/mL can be applied to any undiluted swab sample which gives an absorbance value which falls within the quantifiable range (0.5–10 ppm).

Because of the variability of the swabbing process, the amount of detectable protein in the swabbing solution may vary considerably. Recoveries of various allergens from a swabbed area vary quite widely and detection of allergens/egg from complex and/or highly processed food sources can be difficult. For further information see the BioKits Allergen Swabbing Kit insert.

SHELF LIFE

Diluted wash buffer: Once diluted 10-fold, the wash buffer is stable at room temperature in a sealed clean container for at least 1 week.

Diluted assay diluent: Fresh assay diluent should be prepared for each assay.

Extraction buffer: Fresh extraction buffer should be prepared daily.

Extracted samples: The undiluted sample extracts may be stored at 2–8ºC (35–46°F) for up to five days. If prolonged storage is required the undiluted extracts must be kept frozen (< 20ºC) where they are stable for several months.

Kit reagents: The kit should be stored at 2–8ºC (35–46°F). The shelf life of unopened kit components is indicated by the expiry date on the respective labels. Once the kit reagents have been opened, exposure to elevated (i.e. room) temperatures should be minimized.

Antibody sensitized microwell module must be kept dry. Keep sealed in foil pouch with desiccant.

Providing these instructions are complied with the opened kit reagents should be stable for many weeks or months at 2–8ºC (35–46°F).

PERFORMANCE CHARACTERISTICS

- The assay is designed to give optimum performance at ambient temperature (18–22ºC; 64–72°F).
- Zero standard wells should be preferably < 0.2 absorbance units.
- Standard S5 OD450 nm preferably >1.5 absorbance units.
- Egg spike control should read off the curve within the specifications printed on the certificate of analysis that accompanies this kit.
- At temperatures below 18ºC or above 22ºC incubations may need to be lengthened or reduced respectively to maintain performance.

Poor replication (e.g. six zero standard wells %CV >12.5–15%) may indicate inadequate washing, contamination of the TMB substrate or splashing of Avidin peroxidase. Such imprecision is an indication of a problem during the performance of the assay, which may be INVALID and need to be repeated.

In validation trials, the assay was tested against a panel of commodities (nuts, pulses, grains, proteins, etc.) to check for cross-reaction and interference. Most gave NEGATIVE responses in the assay. Hazelnut, cocoa powder and plain chocolate samples extracted, diluted and assayed as described above returned values close to the lower LOQ (0.5 ppm EWP). It is recommended that such samples are diluted 1/20 (1:19) prior to testing (note quantitation range would therefore be 1–20 ppm EWP). An Egg Assay Kit validation report is available on request.

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Service can be reached between 8 a.m. and 6 p.m. Eastern time by calling 800/234-5333 or 517/372-9200 and asking for a Neogen sales representative or Technical Services. Assistance is available on a 24-hour basis by calling 800/234-5333. Training on this product, and all Neogen test kits, is available.

MSDS INFORMATION AVAILABLE

Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's Food Safety test kits, at www.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

WARRANTY

Neogen Corporation makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

TESTING KITS AVAILABLE FROM NEOGEN

Natural Toxins
- Aflatoxin, DON, Ochratoxin, Zearalenone, T-2/HT-2 Toxins, Fumonisin, Histamine

Foodborne Bacteria
- *E. coli* O157:H7, *Salmonella*, *Listeria*, *Listeria monocytogenes, Campylobacter, Staphylococcus aureus*

Sanitation
- ATP, Yeast and Mold, Total Plate Count, Generic *E. coli* and Total Coliforms, Protein Residues

Food Allergens
- Peanut, Milk, Egg, Almond, Gliadin, Soy, Hazelnut, Mustard, Sesame, Shellfish, Walnut

Genetic Modification
- CP4 (Roundup Ready®)

Ruminant By-products
- Meat and Bone Meal, Feed

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