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Cat.# K361 **Total Egg Antigen EIA**

Format version 909

1. Intended use

Egg proteins are among the eight major food allergens. Since 2004, the Food Allergen Labeling and Consumer Protection Act (FALCP) requires mandatory labeling of potential presence of this allergen in foods. Some vaccines against viral diseases are manufactured using the natural antigen mass grown in chicken embryos, and final vaccine preparations may also contain traces of egg proteins. Total Egg Antigen EIA is based on antibody pair recognizing heat resistant epitope of total egg antigen which is present in both egg white and egg yolk. The kit is designed for quantitative measuring of egg antigens in mixed and heat processed foodstuff and vaccines.

2. Principle of the test

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific antibodies detecting total egg antigen. Antigens from the specimen binds to the antibodies fixed on the microwell surface. Unbound material is removed by washing procedure. Second antibodies directed towards another epitope of total egg antigen labeled with peroxidase enzyme, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

3. Performance characteristics.

3.1. Specificity. High specificity of the test is provided by capture monoclonal antibody to total egg antigen from hen's egg only

Tested material, fresh and cooked	Crossreactivity, %
hen's eggwhite	100%
hen's yolk	100%
Chicken (meat)	3%
duck's egg or meat	<1%
quail's egg or meat	<1%
ostrich's or meat	5%
pearl-hen's egg or meat	<1%

3.2. Repeatability. CV for the same sample within one run is not more than 8%.

3.3. Linearity. Results obtained for serial dilutions were linear (90-110% of the pre-calculated concentrations) within the range of 10-300 U/ml.

3.4. Precision. This parameter was estimated by testing of a mixed sample (calibrators 10 and 300 U/ml, 1:1). The obtained results were within 90-110% of the due value (155 U/ml).

3.5. Analytical sensitivity. The limit of quantification, or analytical sensitivity of the test is not more than 5 U/ml that approximately corresponds to 0.005% w/w of egg content in product.

3.6. Clinical sensitivity. To assess the relevance of Total Egg EIA for clinical practice, serial dilutions of commonly used egg white allergens for *in vivo* testing of suspected allergic patients (skin prick test) were tested in the present assay. Allergen preparations from single lots of 3 randomly selected manufacturers turned positive in present test in maximal dilutions (titers) 1:8,000-1:16,000.

4. Kit contents

	Code	Description	Qty	Units	Colour code
1	P361	Total Egg Antigen EIA strips, 8x12 wells covered by pAb to Total Egg Antigen	1	pcs	
2	N003	Plate sealing tape	1	pcs	
3	S011Z	Blue EIA buffer, 50 ml	1	pcs	blue
4	C361Z	Calibrator set, 1 ml each *	1	pcs	purple
5	S008Z	Washing solution concentrate 21x, 22 ml	1	pcs	colourless
6	T361Z	Conjugate, 11 ml	1	pcs	red
7	R055Z	Substrate solution (TMB) , 11 ml	1	pcs	colourless
8	R050Z	Stop solution, 11 ml	1	pcs	colourless
10	K361I	Instruction Total Egg Antigen EIA	1	pcs	
11	K361Q	QC data sheet Total Egg Antigen EIA	1	pcs	
*	The set contains 5 calibrators: 0, 10, 30, 100, 300 U/ml of Total Egg Antigen				

5. Materials required but not provided

- Microplate reader equipped with 450 nm filter and measuring within the range of 0-3.0 OD units.
- Incubator for 37°C
- Distilled or deionized water
- Preservative for samples (XEMA Cat. # S075Z) – optional
- Balance with precision of 0.1 g (for weighting products)

6. Storage and handling notes

1. Do not mix or use components from kits with different lot numbers.
2. Replace caps on reagents immediately. ATTENTION: Do not swap caps.
3. All kit components should be stored at +2 - +8°C.
4. After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.
5. Do not use wash solutions from other kits (e.g., those with AP conjugates) which may contain sodium azide. Even trace amounts of sodium azide significantly inhibit activity of peroxidase, thus leading to a dropped signal.
6. ATTENTION! During incubations (except for that with TMB) the strips should be tightly sealed with sealing tape. Also avoid drying of wells between incubations.
7. It is recommended to assay all calibrators and unknown samples in duplicates.
8. Wells washing between incubations may be made both manually and with automatic plate washers. In any case, at least 250 µl/well of washing solution should be added during each wash cycle. No soaking between wash cycles is necessary. For manual washing, the procedure should be finalized by dropping out residual liquid from wells onto absorbent paper.
9. OD values should be measured within 15 minutes after stopping TMB color reaction

7. Reagents preparation

1. Before opening, bring the whole kit to room temperature during 30 minutes.
2. Washing solution: empty the vial with Washing solution, 21x concentrate (S008Z, 22 ml) into a 500 ml volumetric flask, add 440 ml of distilled water and mix thoroughly. In case of partial use, dilute Washing solution concentrate (S008Z) 1:21 in distilled water – e.g., 1 ml concentrate + 20 ml distilled water. *Stability after dilution:* 5 days at 18-25°C or 30 days at 2-8°C.

8. Sample preparation for different sample types

An isotonic buffer solution with neutral pH (e.g., 0.1 M phosphate buffer with 0.15 M NaCl) should be used for sample preparation (extraction buffer). If extracts should be stored for more than 24 hours, it is recommended to add a preservative (e.g., sodium azide in 0.1% final concentration). We recommend to use our special Sample Preservative (Cat. # S075Z) which may be ordered separately.

For preparation of some sample types, the following disposables are required:

- cotton swabs (e.g., ear swabs)
- plastic spatula (e.g., those used for mixing of beverages)
- disposable blade or scalpel
- plastic tubes with screw caps for 15-50 ml (e.g., Sarstedt, Cat.# 60.732.001)

ATTENTION: for all sample manipulations, only disposable materials should be used. For bulky objects, sampling should be made in disposable gloves which should be changed for EACH OBJECT.

Sample type	Sample preparation method	Measuring units
Hard and soft food products	Using a disposable plastic spatula, take a sample, put 1.0 g of it into a pre-weighted sampling tube. Add 10 ml of extraction buffer, close the tube with a screw cap and mix thoroughly (either by inverting or by vortexing). When testing bigger industrial samples, it is recommended to take a mixed sample of 10 g from different locations, put it into a disposable flask and add 100 ml of extraction buffer. In this case, mixing should be made with disposable spatula.	U/ml

Liquid foodstuff or vaccine preparations may be tested directly if do not contain solid particles or fat.

The extracts prepared as shown above should be cleared from particles (by sedimentation for NLT 2 hrs, or by short (3-5 min) centrifugation at 300-500 g, or by filtering through a gauze tampon or paper filter. The cleared sample should be assayed immediately or stored at +2 - +8°C for not more than 24 hrs. If XEMA Sample Buffer (Cat.# S075Z) is used, the cleared sample may be stored at +2 - +8°C up to 7 days. For longer storage, samples should be stored frozen at -20°C or lower; in this case, samples may be stored up to 1 year. ATTENTION: avoid repeated thawing-freezing, as this may lead to unpredictable decrease in concentration of the detected antigen. After thawing, the sample should be cleared again (see above) as freeze-thaw cycle may lead to formation of aggregated particles.

9. Test flowchart

1	Put the desired number of microstrips into the frame; allocate 10 wells for the calibrators and two wells for each unknown sample.
2	If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using reagent S011Z (Blue EIA buffer). Use of other buffers or reagents for sample dilution may lead to incorrect results.
3	Pipet 100 µl of calibrator or unknown samples extracts into the wells.
4	Incubate 30 minutes at 37°C.
5	Prepare Washing solution by 21x dilution of Washing solution concentrate (code S008Z) by distilled water. Diluted Washing solution is stable for 30 days at +2-8°C. Wash strips 3 times
6	Dispense 100 µl of Conjugate into the wells.

7	Incubate 30 minutes at 37C.
8	Wash the strips 5 times.
9	Pipet 100 ul of Substrate into the wells
10	Incubate 15 minutes at 20-25 C
11	Pipet 100 ul of Stop solution into the wells.
12	Measure OD (optical density) at 450 nm.
	Set photometer blank on first calibrator
*	Apply point-by-point method for data reduction.

See the example of calibration curve in Quality Control data sheet

Expected values

Due to the regulations of some state governments, the processed food is declared as NOT containing hen's egg proteins only if it contains less than 1% hen's egg proteins by weight. As "egg allergen free" may be marked products that show concentration below analytical sensitivity of the kit.