

Document: K384IE

Instruction version: 009

XEMA Co., Ltd.

P.O.Box 58, 105043 Moscow, Russia

Telephone/fax +7 (495) 737-39-36; 737-00-40 email info@xema-medica.com internet www.xema-medica.com

Cat.# K384 **Soybean antigen EIA**

Format version 909

1. Intended use

Soybean antigen EIA is based on monoclonal antibody pair recognizes heat sensitive epitopes of soybean globulin. The kit is designed for quantitative measuring of percentage content of soybean derivatives in processed meat products. The kit may be also used to detect semi-quantitatively the massive adulterations of mixed and/or processed foods by soy proteins.

Due to its low sensitivity this kit is NOT recommended for detection of the trace amounts of soy derived proteins required by the patients with allergy to soy. Please refer to XEMA SBTI EIA kit (cat # K384T) for this purpose.

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 common soybean globulin 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 soybean 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 monoclonal antibodies to soybean antigen (soybean globulin).

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 1-50 %.

3.4. Precision. This parameter was estimated by testing of a mixed sample (calibrators 5 and 50 U/ml, 1:1). The obtained results were within 90-110% of the due value (22,5 %).

3.5. Analytical sensitivity. The limit of quantification, or analytical sensitivity of the test is not more than 1 % w/w for sausages, 3% for canned meat products (see "Sample preparation for different sample types").

4. Kit contents

	Code	Description	Qty	Units	Colour code
1	P384	Soybean antigen EIA strips, 8x12 wells covered by mAb to soybean antigen	1	pcs	
2	N003	Plate sealing tape	1	pcs	
3	SP384Z	EIA sample buffer, 50 ml	1	pcs	blue
4	C384Z	Calibrator set, 1 ml each *	1	pcs	blue
5	S008Z	Washing solution concentrate 21x, 22 ml	1	pcs	colourless
6	T384Z	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	K384I	Instruction Soybean antigen EIA	1	pcs	
11	K384Q	QC data sheet Soybean antigen EIA	1	pcs	
*	The set contains 6 calibrators: 0, 1, 5, 15, 25, 50 % of soybean 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 microliter 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	Recalculation
Sausage,bacon	Using a disposable plastic spatula, take a sample, put ca. 1.0 g of it into a pre-weighted sampling tube, weigh the sample and determine net weight of the sample. 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. Dilute extract with EIA Sample buffer 1:40, for example 25 µl of extract and 975 µl of buffer. Add 100 µl into microplate wells	%	No
Canned meat		%	Value obtained from calibration curve should be multiplied by 3 to obtain actual soy % in product
Other hard and soft food products		QUALITATIVE	No

For all the above sample preparations, extracts 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 12 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 SP384Z (EIA Sample buffer). Use of other buffers or reagents for sample dilution may lead to incorrect results.
3	Pipet 100 ul of calibrator or unknown samples into the wells.
4	Incubate 30 minutes at 37C.
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 +2-8C. Wash strips 3 times
6	Dispense 100 ul 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. **For some sample types, the obtained results should be recalculated** (see sec. [8. Sample preparation for different sample types](#)).

Expected values

Due to the regulations of some state governments, the processed meat is declared as NOT containing soybean proteins only if it contains less than 1% soy proteins by weight, i.e. the quantity which shows undetectable value in a present assay