

Document: K826E Instruction version: 011
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Cat.# K826 Alternaria antigen EIA Format version 801

Summary

Alternaria is widely distributed genus of fungi. Dead plant remains is natural reservoir of *Alternaria*, from this source this fungus invades in soil. *Alternaria* is always present on seeds from different plants and influences on seed germination. *Alternaria* induces diseases of different agricultural crops. *A. tenuis* induces spottiness of cotton and citrus plants; *A. brassicae* affects cabbage, pepper, aubergines. *A. dauci* affects carrot, *A. solani* – potatoes and tomatoes. *A. tenuis* produces tentotoxin that inhibits production of chlorophyll.

Alternaria is widely distributed among cereals – in wheat, in rye, in barley. *Alternaria* antigens may remain and accumulate in foods during food processing, especially if no high temperature heating is used. Besides of cereals, *Alternaria* also colonizes processed fruit and vegetables, eg juices, jams and tomato paste. The presence of *Alternaria* antigens in food may cause allergic symptoms. Spores of *Alternaria* is capable to induce chronic rhinoconjunctivitis or bronchial asthma after inhalation.

Fungi from genus *Alternaria* can survive on different synthetic substrates and cause damage of furniture and clothes. *Alternaria* antigens are also detected in conditioning systems and refrigerators, because low temperature and high humidity create favorable conditions.

Intended use

The kit is intended for quantitative determination of *Alternaria alternata* and relative species antigens in grain, food, washes from industrial equipment and other types of material and allows 96 determinations or assaying of 42 samples in duplicates.

Principle of the test

This test is based on competitive immunoassay principle. Tested specimen is placed into the microwells coated by the antigens of *A. alternata* simultaneously with polyclonal rabbit IgG to *Alternaria spp.* antigens. Antigen from the specimen binds to the rabbit IgG in competition with antigen on the microwells. Unbound material is removed by washing procedure. Second antibodies directed towards rabbit IgG and labelled 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 inversely proportional to the quantity of antigen in the specimen.

KIT CONTENTS

	Code	Description	Qty	Units	Color code
1	P826	<i>Alternaria</i> antigen EIA strips, 8x12 wells, breakable	1	pcs	0
2	N003	Plate sealing tape	1	pcs	0
3	S011Z	Sample extraction/dilution EIA buffer, 53 ml	4	pcs	blue
4	C826Z	Calibrator and control set, 1,0 ml	2	pcs	dark blue
5	AS826Z	Rabbit IgG to <i>Alternaria spp.</i> Antigens, 5,5 ml	1	pcs	dark blue
6	S008Z	Washing solution concentrate 21x, 22 ml	1	pcs	0
7	T301Z	Swine polyclonal antibodies to rabbit IgG conjugated to HRPO, 11 ml	1	pcs	dark blue
8	R055Z	TMB substrate solution, 11 ml	1	pcs	
9	R050Z	Stop solution, 11 ml	1	pcs	
10	K826IE	Instruction <i>Alternaria</i> antigen EIA, English	1	pcs	
11	K826Q	QC data sheet <i>Alternaria</i> antigen EIA	1	pcs	

Materials required but not provided

- Distilled or deionized water;

NECESSARY EQUIPMENT.

1. Microplate photometer with 450 nm wavelength and OD measuring range 0-3.0.
2. Dry thermostate for 37°C
3. Analytical balancing device with precision ≤ 0.05 g.

HANDLING NOTES

1. Reagents remain stable within 1 month after reconstitution.
2. Do not mix and/or use reagents from different lots within one run.
3. Replace caps on reagents immediately. Do not swap caps.
4. All kit components should be stored in the freezer (at +2 - +8°C). Do not freeze the kit or its components!
5. After opening the pouch keep unused microtiter wells **TIGHTLY SEALED BY adhesive PLATE SEALING TAPE (INCLUDED)** to minimize exposure to moisture.
6. Do not use washing solutions containing sodium azide – even in trace quantities, it inhibits peroxidase, thus reducing color development.
7. Attention: during all incubations, please, seal the wells with adhesive tape, Do not allow drying of wells between assay steps.
8. It is recommended to assay all samples, calibrators and controls in duplicates.
9. Washing of wells may be made either manually or with automatic washing device. During each wash cycle, dispense nlt 250 ul of Washing solution into each well. Soaking is not required. If washed manually, please, shake out the residual Washing solution from the wells by tapping on filter paper.
10. Please, measure OD in the wells within 15 minutes after addition of stop solution.

ASSAY PROCEDURE**Reagent preparation**

All reagents (including the required number of strips) should be brought to RT (20-25°C) before use.

Sample preparation:

*1	Crush a food or grain sample. Use blender or mortar to crush solid products. Continue crushing until a homogeneous powder is obtained. If liquid products are analyzed, mix them thoroughly in the original packaging. Attention! If different products are analyzed, wash your crushing device thoroughly to avoid cross-contamination!
*2	Extract antigens from the samples to be analyzed. To do this, mix the pulp obtained or liquid sample with buffer S011 (included into the kit) 1:10 (W/V) – e.g., 1 g + 10 ml of S011.
*3	Incubate 30 min at 20-25C with periodical shaking.
*4	Centrifuge extracts 5 min at 200 g to eliminate particulate matter. Use supernatant for further analytical steps.

Assay run:

*5	Put the desired number of microstrips into the frame; allocate two wells for each unknown sample and 12 wells for the calibrators and control samples.
*6	Pipet 50 ul of calibrator, control or unknown sample extract into the respective wells. Attention! The order of reagents addition should be strictly followed!
*7	Pipet 50 ul of rabbit IgG to <i>Alternaria</i> antigens (AS826Z) in all wells. Mix the contents carefully by gentle shaking of the plate and seal the wells with adhesive tape.
*8	Incubate 60 min at 37°C.
*9	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
*10	Dispense 100 ul of Conjugate into the wells.

*11	Incubate 30 minutes at 37°C
*12	Wash the strips 5 times.
*13	Pipet 100 ul of Substrate into the wells
*14	Incubate 10-20 minutes at 18-25°C
*15	Pipet 100 ul of Stop solution into the wells.
*16	Measure OD (optical density) at 450 nm.
*17	Set photometer blank on first calibrator
*18	Use point-by-point method for data reduction

Examples of calibration curves are given in the QC sheet.

Calculation coefficient:

Content of *Alternaria antigens (µg/g of product) = Concentration of *Alternaria* antigens (directly from calibration curve), µg/ml × 10****

* Test was calibrated by *Alternaria alternata* antigens, the absolute value of antigens from other species may be different (+-15-20 % variation was obtained in preliminary studies).

** Volume of extract obtained from 1 g of a product analyzed.

Attention! The range of *Alternaria* infection may be variable in different geographical zones. GLP rules recommend that each laboratory should establish its own reference range.

Quality control

Control sample(s) should fall into the ranges shown in QC insert (see attached).

Expected values and normal range

	Lower limit	Upper limit
	µg/g	
<i>Alternaria</i> antigen-free products	0	15