

# Miagnostic Automation/Cortez Diagnostics, Inc.



AccuDiag™ IgG4 Food Antigen Screen

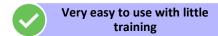
REF 5155-8



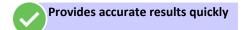
**ELISA Kit** 

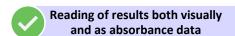
IgG4 Food Antigen ELISA			
Principle	Indirect ELISA		
Detection	Qualitative		
Sample	10 μL serum/plasma		
Incubation Time	150 minutes		
Shelf Life	12 Months from the manufacturing date		

#### **PRODUCT FEATURES**









### **INTENDED USE**

Diagnostic Automation, Inc. (DAI)  $IgG_4$  Screen 88 Food Antigens ELISA Test Kit has been designed for the detection and the quantitative determination of specific  $IgG_4$  antibodies against food antigens in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of DAI.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

#### SIGNIFICANCE AND SUMMARY

Incompatibility reactions against food may cause various symptoms in the human organism and this disturbance is manifested in the immune system by the formation of specific IgE, IgG or IgG $_4$  antibodies.

Statistics show that 60% of the population suffer from intolerances against at least one foodstuff, which may cause clinical symptoms or enhance them. Hints may be various and reach from skin irritations over digestive disorders up to migraine. With the diagnostic findings of unspecific discomfort, allergies or intolerances against food should be clarified.

The theoretical basis for the determination of specific IgG or IgG $_4$  for the diagnosis of food intolerances depends on the observation that some subclasses of IgG (mainly IgG $_4$ ) are connected to the in vitro degranulation of basophilic cells and mastocytes and the activation of the complement cascade. It was also observed that high concentrations of circulating IgG were measured in atopic persons.

Already early surveys showed that in persons with inflammatory reactions against food IgG but not IgE was detected. Significantly enhanced IgG and IgG $_4$  titers were also found in patients with food intolerances.

Skin tests are relatively poorly correlated to food allergies and are only significant in the presence of IgE related reactions. As additional diagnostic tools provocation and elimination diets are applied. These methods depend strongly on the motivation and compliance of the patient. Due to these constraints nowadays serological determinations of antibodies against various food panels are applied increasingly.

The two reactions related with the immune system differ insofar as the IgE associated food allergy occurs within the next hour following the food intake, while  $IgG/IgG_4$  intolerances show a delayed reaction of 24 to 120 hours and persistent symptoms may arise.

## **ASSAY PRINCIPLE**

The DAI IgG<sub>4</sub> Screen 88 Food Antigens ELISA test kit is based on the principle of the enzyme immunoassay (EIA). 88 different food antigens and 8x reference antigens (egg white) for standards and controls are bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards and controls are pipetted into the wells of the microtiter plate. A binding between the IgG<sub>4</sub> antibodies of the serum and the immobilized antigens takes place. After a one hour incubation at 37°C, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG<sub>4</sub>-AP conjugate is added and incubated for 30 minutes at 37°C. After a further washing step, the substrate (PNPP) solution is pipetted and incubated for 60 minutes at 37°C, inducing the development of a yellow dye in the wells. The color development is terminated by the addition of a stop solution. The resulting dye is measured spectrophotometrically at the wavelength of 405 nm. The concentration of the IgG<sub>4</sub> antibodies is directly proportional to the intensity of the color.

#### SPECIMEN COLLECTION AND PREPARATION

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 7 days. For a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 100  $\mu L$  serum + 10 mL sample diluent).

# **REAGENTS**

#### Materials provided with the kit

The  $IgG_4$  Screen 88 Food Antigens ELISA kit contains sufficient reagents for one patient (88 determinations). The first strip of the plate contains reference

Diagnostic Automation/Cortez Diagnostics, Inc.

21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax: 818-591-8383

Email: onestep@rapidtest.com Website: www.rapidtest.com

5155-P1 Page 1 of 4

# Miagnostic Automation/Cortez Diagnostics, Inc.



antigen (egg white, for) for the generation of a calibration curve and the

determination of controls.

1. Food/Reference antigen coated microtiter Plate (1)

- 2. Standards: 0.35, 0.70, 3.5, 17.5, 100 U/ml (0.5 ml each)
- 3. Controls: Weak positive, Strong positive (0.5 ml each)
- 4. Anti-human IgG4 Enzyme Conjugate (15 ml)
- 5. Substrate (15ml)
- 6. Stop Solution (15ml)
- 7. Sample Diluent (40 ml)
- 8. Washing Buffer (10x) (60ml)

#### **Universal Reagents**

- Sample diluent, washing buffer and stop solution are identical for all IgG<sub>(4)</sub> food antigens screen test kits from DAI with Alkaline Phosphatase as detecting enzyme and may be interchanged between products and lots. All other reagents are assigned to a special kit lot and must not be mixed.
- Microtiter Plate-1 microtiter plate, coated with 88 food antigens (see distribution scheme) and 8x reference antigen (1. strip, color coded black). Ready-to-use.
- Standards A-F- 6 x 0.5 mL, human plasma diluted with PBS/BSA, with 0.35, 0.70, 3.5, 17.5, 50 and 100 U/mL of IgG antibodies against egg white (f1). Addition of 0.05% sodium azide. Ready-to-use.
- Weak Positive Control- 0.5 mL, human plasma diluted with PBS/BSA, including low concentrations of IgG antibodies. Addition of 0.05% sodium azide. Ready-to-use.
- Strong Positive Control- o.5 mL, human plasma diluted with PBS/BSA, including high concentrations of IgG antibodies. Addition of o.05% sodium azide. Ready-to-use.
- Anti-human-IgG<sub>4</sub> Enzyme Conjugate- 15 mL, mouse-a-human-IgG<sub>4</sub>-AP, in proteinaceous buffer solution. Addition of o.o1% methyl-isothiazolone, o.o1% bromonitrodioxane and 5 mg/L ProClin. Ready-to-use.
- Substrate- 15 mL, PNPP (Paranitrophenylphosphate). Ready-to-use.
- Stop Solution- 15 mL, 1 M sodium hydroxide. Ready-to-use.
- Sample Diluent- 40 mL, PBS/BSA buffer. Addition of 0.05% sodium azide. Ready-to-use.
- Washing Buffer- 60 mL, PBS + Tween 20, 10x concentrate. Final
  concentration: dilute 1+9 with deionized water. If during the cold storage
  crystals precipitate, the concentrate should be warmed up at 37°C for 15
  minutes.

## Materials required but not provided

- 1. 100 μL and 1000 μL micro- and multichannel pipettes
- 2. Microtiter Plate Reader (405 nm)
- 3. Microtiter Plate Washer
- 4. Reagent tubes for the serum dilution
- 5. Deionized water

### **REAGENT PREPARATION**

**Washing Solution:** dilute before use 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

# **ASSAY PROCEDURE**

- 1. For each patient sample prepare one microtiter plate.
- 2. Pipet 100  $\mu$ L each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells (see distribution scheme).
- 3. Cover plate and incubate for 60 minutes at 37°C.
- 4. Empty the wells of the plate (dump or aspirate) and add 300 μL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
- Pipet 100 μL each of ready-to-use conjugate into the wells.
- Cover plate and incubate for 30 minutes at 37°C.
- Empty the wells of the plate (dump or aspirate) and add 300 μL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
- 8. Pipet 100 μL each of the ready-to-use substrate into the wells.
- 9. Cover plate and incubate for 60 minutes at 37°C.
- 10. To terminate the substrate reaction, pipet 100  $\mu L$  each of the ready-to-use stop solution into the wells.
- 11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 405 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

## **TROUBLESHOOTING**

Negative control has excessive color after development.

Reason: inadequate washings.

**Correction:** wash more vigorously. Remove excessive liquid from the wells by tapping against an absorbent towel. Do not allow test wells to dry out.

### **RESULTS**

The evaluation can be performed either in units per mL (U/mL) or in classes. Example:

Standard	Class	OD-Value
0.35 U/mL	1	0.156
0.7 U/mL	2	0.212
3.5 U/mL	3	0.445
17.5 U/mL	4	1.035
50 U/mL	5	1.660
100 U/mL	6	2.569

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

#### **Quantitative Evaluation**

The ready-to-use standards and controls of the  $IgG_4$  Screen 88 Food Antigens ELISA test kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. The values for controls and standards in units are printed on the labels of the vials.

For a quantitative evaluation the absorptions of the standards are graphically drawn *point-to-point* against their concentrations. From the resulting reference curve the concentration values or the respective reaction class for controls and each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. As curve fit *point-to-point* has to be chosen.

Diagnostic Automation/Cortez Diagnostics, Inc.

21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax: 818-591-8383

Email: onestep@rapidtest.com Website: www.rapidtest.com

5155-P1 Page 2 of 4



# Diagnostic Automation/Cortez Diagnostics, Inc.



# IVI IVI O IV O D I

## PERFORMANCE CHARACTERISTICS

IgG₄ ELISA	Egg White	Cow's Milk	Tomato
Intra-Assay- Precision	7.7%	8.0%	8.7%
Inter-Assay- Precision	6.6 – 10.9%	8.4 – 13.0%	4.6 – 7.4%
Inter-Lot-Precision	2.5 - 11.4%	5.6 – 11.8%	0.5 – 9.6%
Analytical Sensitivity	0.22 U/mL	0.17 U/mL	0.16 U/mL
Recovery	90 – 107%	89 – 103%	87 – 97%
Linearity	82 – 114%	73 – 100%	102 – 120%
Cross-Reactivity	No cross reactivity towards IgE up to 100000 IU/mL.		
Interferences	No interferences with bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL.		
Clinical Specificity	88%	86%	90%
Clinical Sensitivity	86%	94%	80%

# **INSTRUCTION CHART**

1.	Reaction: Serum	100 μL	Standards/Controls (ready-to-use) Patient serum (diluted 1:101)		
60 min <b>√</b> 37°C					
		Wash, 3x, 300 µL washing buffer (diluted 1:10)			
2.	Reaction: Conjugate	100 µL	Conjugate (ready-to- use)		
			30 min <b>V</b> at 37°C		
		Wash, 3x, 300 µL washing buffer (diluted 1:10)			
<u> </u>					
3.	Reaction: Substrate	100 µL	Substrate (PNPP, ready-to-use)		
	60 min <b>√</b> at 37°C				
		100 µL	Stop solution (NaOH, ready-to-use)		

#### **PRECAUTIONS**

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety
  precautions as well as the prohibition of eating, drinking and smoking in the
  lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless, precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25°C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.

- 6. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- 7. When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- 8. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- 9. All reagents have to be used within the expiry period.
- 10. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- 11. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there is a danger of intoxication.

### STORAGE CONDITIONS

Store kit components at 2-8°C. After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

#### **REFERENCES**

- Aas K: The diagnosis of hypersensitivity to ingested foods. Clinical Allergy 1978; 8:39-50.
- AMA Council on Scientific Affairs, In Vitro Testing for Allergy. Report II of the Allergy Panel Council on Scientific Affairs. JAMA, 1987, 258(12):1639-43.
- AMA Council on Scientific Affairs, In Vivo Diagnostic Testing and Immunotherapy for Allergy. Part I, JAMA, 1987, 258:1363-7.
- 4. Bleumink E: Food Allergy; the chemical nature of the substance eliciting symptoms. World Reviews in Nutrition and Diet 1970; 12:505-570.
- Bübl, R. Schön, B., Rakoski, J.: Allergenspezifische IgG-Antikörper bei Atopikern; Allergologie 16, 7, 299-304 (1993).
- Canadian Paediatric Society, Allergy Section. Blood Tests for Allergy in Children. Can Med Assoc J, 1990, 142(11):1207-8.
- Cohen, G.A., Hartmann, G., Hamburger, R.N., O'Connor, R.D.: Severe anemia and chronic brochitis associated with a markedly elevated specific IgG to cow's milk protein; Annals of Allergy 55, 38-40 (1985).
- 8. Devey, M.E., Wilson, D.V., Wheeler, A.W.: The IgG subclass of antibodies to grass pollen allergens produced in hay-fever patients during hyposensitization; Clin. Allergy 6, 227 (1976).
- Durham, S.R., Lee, T.H., Cromwell, O., Shaw, R.J., Merret, T.G., Merret, J., Cooper, P, Kay, A.B.: Immunologie studies in allergen-induced late-phase asthmatic reactions; J. Allergy Clin Immunol 74, 49 (1984).
- Djurup, R., Osterballe, O.:IgG subclass antibody response in grass pollenallergic patients undergoing specific immunotherapie; Allergy 39, 433-441(1984).
- 11. Rowntree, S., Platt-Mills, T.A.E, Cogswell, J.J, Mitchell E.B.: A subclass IgG4-specific antigen-binding Radioimmunoassay (RIA): Comparison between IgG and IgG4 antibodies to food and inhaled antigens in adult atopie dermatitis after desensitization treatment and during development of antibody responses in children; J.Allergy Clin. Immunol 80, 622-630 (1987).
- Shakib, F., McLaughlan, P., Stanworth, D.R., Smith, E., Fairburn, E.: Elevated serum IgG and IgG4 in patients with atopic dermatitis; Br. J. Derm: 97, 59-63 (1977).
- 13. Wojdani, A., Etessami, S., Cheung, G.P.:IgG is not the only inhibitor of IgE in the RAST test; Annals of Allergy 55, 463-468 (1985).
- 14. Wüthrich, Brunello: Neurodermitis atopica (atopische Dermatitis) in Fuchs/Schulz, Manuale Allergologicum V, 14, 21-22.

Diagnostic Automation/Cortez Diagnostics, Inc.

21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax: 818-591-8383

Email: onestep@rapidtest.com Website: www.rapidtest.com

5155-P1 Page 3 of 4





# **MANUFACTURER AND BRAND DETAILS**



5155-P1 Page 4 of 4