11. Assay Procedure Continued…

8. Add 100µl of Stop Solution **(Reagent 5)** to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.
9. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

12. Quality Control

The expected OD values and the acceptance ranges for the Standards and the Positive Control are given on the certificate included in the kit.

The Positive Control is intended to monitor for substantial reagent failure.

Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

13. Interpretation of Results

Plot the OD of the 0 and 25 U/ml Standard against concentration and draw a straight line through the points. Read the unknowns off this curve. The following table gives suggested concentration ranges and grades for different food antibody responses:

Response	Range (AU/ml) [1]	Grade
Negative	<8	0
Borderline	8 - 12.5	1 (Equivocal)
Positive	12.5 - 25.0	2+
Strong positive	>25.0	3+

[1] *Units are arbitrary Genesis units.*

These are suggested ranges, based on in-house studies at Genesis Diagnostics Ltd. Users of the kit should verify these ranges in their own laboratory under local conditions.

14. Limitations of the Procedure

Results must always be correlated to the clinical condition of the patient, since a raised food IgG level need not manifest as any specific symptoms.

It should be noted that results from this kit give no information about IgE mediated allergy.

15. Performance Characteristics

Between plate imprecision < 20%

16. 40 Food IgG – Food Antigen Layout

The microplate contains 12 strips of 8 wells, which are coded:

4 strips are coded #1
4 strips are coded #2
4 strips are coded #3

For each patient use a set of 1-2-3. The table below shows the locations of the different food antigens.

Strip 1	Food Antigen	Strip 2	Food Antigen	Strip 3	Food Antigen
A1	*Anti-food IgG Standard 0 U/ml*	A2	Cow's Milk	A3	Gluten
B1	*Anti-food IgG Standard 25 U/ml*	B2	Egg White	B3	Apple & Pear
C1	*Anti-food IgG Positive Control*	C2	Egg Yolk	C3	Berries Mixture (Raspberry, Strawberry, Blackberry)
D1	Corn	D2	White Fish Mixture (Cod, Haddock, Plaice)	D3	Citrus Mixture (Orange, Lemon, Grapefruit)
E1	Oat	E2	Shellfish Mixture (Crab, Lobster, Prawn)	E3	Nut Mixture (Almond, Cashew, Hazelnut, Peanut)
F1	Rice	F2	Soya	F3	Yeast (Bakers & Brewer's)
G1	Rye	G2	Legume Bean Mixture (Haricot, Kidney, Pea)	G3	Chicken & Turkey
H1	Wheat	H2	Mustard Mixture (Cabbage, Broccoli, Cauliflower)	H3	Pork & Beef

17. Method Summary

- Add 10µl of Sample to one vial of Diluent **(Reagent 1)**
- Dispense Standards, the Positive Control and the diluted sample into the specified microplate wells
- Incubate for **30 minutes** at room temperature.
- *Wash the wells three times*
- Dispense 100µl of Conjugate **(Reagent 3)** into each well
- Incubate at room temperature for **30 minutes**
- *Wash the wells four times*
- Add 100µl of TMB Substrate **(Reagent 4)** to each well
- Incubate at room temperature for **10 minutes**
- Add 100µl Stop Solution **(Reagent 5)** to each well
- Read the optical density at 450nm (single wavelength) or 450/620nm (dual wavelength).

18. Further Reading

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Eden Research Park, Henry Crabb Road, Littleport, Cambridgeshire,
CB6 1SE, UK Tel+ 44(0)1353 862220 Fax+44(0)1353 863330
Email: support@elisa.co.uk Web: www.omegadiagnostics.com
Certified to ISO9001:2008, ISO13485:2003

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G·E·N·E·S·I·S Diagnostics

Food IgG (40 Foods) ELISA Kit

Semi-quantitative Assay for investigation of IgG-mediated food sensitivity

Product Code: GD015

For *in vitro* Diagnostic Use

CE

1. Materials Included in the Kit

- **Microplate:** 96 well plate pre-coated with different food antigens in a foil bag with desiccant; strips are in sets of three and are number-coded 1, 2, 3
- **Reagent 1**: **Sample Diluent**, 4 vials containing 10mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 4 ml, (blue), ready to use
- **Reagent 2: Wash Buffer** 100mM Tris-buffered saline with detergent, pH 7.2, 100ml, **Concentrate** (X 10)
- **Reagent 3: Conjugate** goat anti-human IgG conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12 ml, (red), ready to use
- **Reagent 4: TMB Substrate** aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- **Reagent 5: Stop Solution** 0.25M sulphuric acid, 12 ml, ready to use
- **Standards:** 0, & 25 U/ml, 10mM Tris-buffered saline containing human serum IgG antibodies, 1 ml, ready to use
- **Positive Control:** 10mM Tris-buffered saline containing human serum IgG antibodies, 1 ml, ready to use
- **Instructions for use**

263-015-05

2. Other Equipment Required

Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 25µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable, self-validated automated system may be used.

Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

3. Intended Use

The Food IgG kit is a rapid ELISA method for the measurement of IgG antibodies to 40 different food antigens, in human sera or plasma. The components of the kit are for *in vitro* diagnostic use only.

4. Explanation of the Test

Many people exhibit chronic food sensitivity reactions to specific food antigens. Unlike the immediate effects of IgE-mediated allergy, IgG-mediated food sensitivity reactions may take several days to appear. Controlled removal of the problem foods from the patient's diet will, in many cases, rapidly improve the patient's condition. General lethargy, weight gain, dermatitis, arthritis and tiredness are associated with food allergies. Irritable bowel syndrome may also be linked to food sensitivity.

5. Principle of the Test

Diluted serum samples are incubated with antigen extracts from 93 different foods immobilised on microtitre wells. After washing away unbound serum components, goat anti-human IgG conjugated to horseradish peroxidase is added to the wells, and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of Stop Solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the standards, positive control and samples are measured using a microplate reader at 450nm.

6. Safety Precautions

1. All reagents in this kit are for *in vitro* diagnostic use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. All human source material used in the preparation of Standards and the Positive Control for this product have been tested and found negative for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the TMB Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local legislation.

7. Technical Precautions

1. The microplate and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use.
3. Include the Positive Control in every test run to monitor for reagent stability and correct assay performance.
4. Strictly observe the indicated incubation times and temperature.
5. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
6. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB substrate.
7. Do not allow microwells to dry between incubation steps.
8. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
9. Avoid direct sunlight and exposure to heat sources during all incubation steps.
10. Replace colour-coded caps on their correct vials to avoid cross-contamination
11. It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

8. Shelf Life and Storage Conditions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer has a shelf life of 3 months if stored in a closed bottle at 2 - 8°C.

9. Specimen Collection and Storage

Serum, plasma or whole blood samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

10. Preparation of Reagents

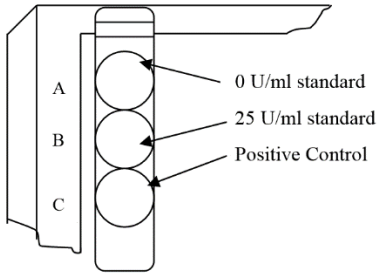
1. Dilute the Wash Buffer **(Reagent 2)** 1: 9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water.

2. Add 10 µl of patient serum to one of the vials of Sample Diluent **(Reagent 1)** provided. Alternatively, 20 µl of whole blood may be used.

11. Assay Procedure

One to four patients may be processed simultaneously. The microplate contains strips numbered 1, 2, & 3. The strips must be used in the correct order, *i.e.* from left to right 1 - 2 - 3. One set of strips is used for each patient. See section16 for the food antigen list. Some wells contain a mixture of related foods. Unused strips can be stored in the foil pouch with drying agent until required.

Important note:
Before starting to dispense samples, ensure that strips are correctly orientated in the frame. The chamfered ends of the strips must be at the top of the frame, as shown below.



1. Dispense 100µl of each Standard and Positive Control into the wells as follows:

Well	Standard/Control
A1	0 U/ml Standard
B1	25 U/ml Standard
C1	Positive Control

2. Dispense 100µl of diluted patient sample into:
   Strip 1: wells D, E, F, G and H,
   Strip 2: wells A, B, C, D, E, F, G and H
   Strip 3: wells A, B, C, D, E, F, G and H
3. Incubate for **30 minutes** at room temperature.
4. After 30 minutes, decant or aspirate the well contents and wash the wells 3 times using automated washing or the manual wash procedure (see below). Careful washing is the key to good results. **Do not allow the wells to dry out.**

   Manual Wash Procedure
   Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with Wash Buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times. Blot the wells on absorbent paper before proceeding. **Do not allow the wells to dry out.**

5. Dispense 100µl of Conjugate **(Reagent 3)** into each well. Incubate the wells for **30 minutes** at room temperature.
6. After 30 minutes, discard the well contents and carefully wash the wells 4 times with Wash Buffer. Ensure that the wells are empty but do not allow to dry out.
7. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate **(Reagent 4)** into each well. Incubate the plate for **10 minutes**. Observe the colour development carefully. The colour development should be homogeneous throughout the well. If any wells show rapid colour development in any single point on the well, it may be due to enzyme-conjugate, which has not been washed away completely. Treat such results with caution.