11. Assay Procedure Continued…

8. After 10 minutes, 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. 5 Food IgG – Strip Layout

The microplate contains 12 strips of 8 wells. One strip is used for each patient.

Well	
A1	Anti-food IgG standard 0 U/ml
B1	Anti-food IgG standard 25 U/ml
C1	Anti-food IgG positive control
D1	Cow's milk
E1	Wheat
F1	Nut mix (almond, hazelnut, peanut, cashew)
G1	White fish mix (cod, haddock, plaice)
H1	Soya proteins

17. Method Summary

- Dilute serum/plasma 1:400
- Use 1 strip for each patient
- 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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G·E·N·E·S·I·S Diagnostics

Food IgG (5 Foods) ELISA Kit

For investigation of food sensitivity to Cow's Milk, Wheat, Nuts, White Fish and Soya

Product Code: GD109

For *in vitro* Diagnostic Use

CE

1. Materials Included in the Kit

- **Microplate:** 96 well microplate coated with cow milk, wheat, nuts, white fish and soya in a foil bag with desiccant.
- **Reagent 1**: **Sample Diluent** 10mM Tris-buffered saline, pH 7.2 with antimicrobial agent, (blue), 50 ml, 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, 12ml, (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, 2 ml, ready to use
- **Positive Control:** 10mM Tris-buffered saline containing human serum, 2 ml, ready to use
- **Instructions for use**

2. Other Equipment Required

Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 10µl, 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 anti-food IgG kit is a rapid ELISA method for the measurement in human sera or plasma of IgG antibodies to cow's milk, wheat, nuts, white fish and soya. Up to twelve patient samples can be tested per kit. Results must always be correlated with the clinical condition of the patient since a raised food IgG level need not manifest as symptoms. It should be noted that results from this kit give no information about IgE mediated allergy.

4. Explanation of the Test

Some people exhibit chronic sensitivity reactions to certain food antigens. Unlike the immediate effects of IgE-mediated allergy, IgG-mediated food sensitivity reactions may take several days to appear.

General lethargy, weight gain, dermatitis, arthritis, tiredness and bowel syndromes may be associated with food sensitivity. Controlled removal of the problem foods from the patient's diet can dramatically improve the patient's condition.

5. Principle of the Test

The kit uses a 96-well microtitre plate format. Extracts from cow's milk, wheat, nuts, white fish and soya are coated onto the surface of the wells. The serum specimen (diluted 1:400) is added to the antigen-coated wells and incubated for 30 minutes to allow food-specific antibodies to bind to the antigens in the microplate wells.

After washing, the specifically bound antibodies are detected using goat anti-human IgG conjugated to horseradish peroxidase. After 30 minutes incubation, unbound conjugate is removed by washing, and TMB substrate is added. A blue colour develops in wells in which food-specific antibodies are present and this changes to yellow upon addition of Stop Solution. The absorbance of the yellow solution is then measured in a microplate reader using 450nm or 450/620nm filters.

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. Dilute the patient sample 1:400 in sample diluent e.g. 5 µl of serum or plasma to 2 ml of Sample Diluent **(Reagent 1)** and mix it well. Alternatively, dilute 10 µl of whole blood in 2 ml of diluent.

11. Assay Procedure

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.

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

Well	Standard/Control
A	0 U/ml Standard
B	25 U/ml Standard
C	Positive Control

2. Dispense 100µl of diluted patient sample into wells 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.