

10. Assay Procedure

1. Load slides into the slide frame holder with the membrane side facing up. The "X" or dot on the slide should be on the bottom left.
2. Using a pipette add 100µl of diluted patient sample (in Sample Diluent) to the pad. Cover and incubate for 30 minutes.
3. Flick off the slide contents, add 120µl of wash buffer and agitate by tapping the side of the frame. Repeat this step 2 more times. Cover and incubate for 5 minutes.
4. Flick off the slide contents, add 120µl of wash buffer and agitate by tapping as in step 3. Cover and incubate for 5 minutes. Flick off the wash buffer. Do not allow the slide to dry.
5. Using a pipette, add 100µl of conjugate to each pad. Cover and incubate for 30 minutes.
6. Flick off the slide contents, add 120µl of wash buffer and agitate by tapping. Repeat this step 2 more times. Cover and incubate for 5 minutes.
7. Flick off the slide contents, add 120µl of wash buffer and agitate by tapping. Cover and incubate for 5 minutes. Flick off the wash buffer. Do not allow the slide to dry.
8. Using a pipette, add 100µl of TMB substrate to each pad. Cover and incubate for 10 minutes.
9. Flick off the slide contents and gently remove slides from the slide frame holder and carefully place into the wash station containing 400ml distilled/de-ionised water for 2 minutes. DO NOT AGITATE.
10. Carefully remove slides and dry in the slide centrifuge for 30 seconds. The microarrays are now ready to scan.
11. Scan using the high resolution flat bed scanner.

11. Quality control

1. The microarrays include positive and negative controls which are intended to monitor for substantial reagent failure.

12. Interpretation of Results

Results are derived from internal IgG standards included in the array.

Response	Range (U/ml) ¹
Negative	<24
Borderline	24 - 30
Positive	>30

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 and adjust as required.

13. Limitations of the Procedure

1. 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.
2. It should be noted that results from this kit give no information about IgE mediated allergy.

14. Assay characteristics

Within assay imprecision <12%
Between assay imprecision <22%

15. 221 Food IgG – Food Antigen Layout

See the food reporting software provided with the kit.

Method Summary	
•	Pipette diluted sample onto the microarray
•	Cover and incubate for 30 minutes at room temperature.
•	Wash the microarrays 3 times and incubate with wash buffer for 5 minutes
•	Wash once again and incubate with wash buffer for 5 minutes
•	Dispense 100µl of conjugate onto each microarray
•	Incubate at room temperature for 30 minutes
•	Wash the microarrays 3 times and incubate with wash buffer for 5 minutes
•	Wash once again and incubate with wash buffer for 5 minutes
•	Incubate with 100µl membrane TMB for 10 minutes
•	Load slides into a wash station containing water and incubate for 2 minutes
•	Dry the slides in a centrifuge for 30 seconds
•	Scan the microarray using a high resolution flat bed scanner and apply associated spot-finding software
•	Process the data using the Foodprint® reporting software

Further reading

Atkinson et al. IgG antibodies in IBS, Gut 2004;53:1459-1464
 James M. Toward an understanding of allergy and in vitro testing. Nat. Med. Journal, 1999; 2 (4): 7-15.
 Gaby AR. The role of hidden food allergy/intolerance in chronic disease. Alt. Med. Review, 1998; 3(2): 90-100.
 Hofman T. IgE and IgG antibodies in children with food allergy. Roc. Akad. Med. Bialmyst, 1995; 40 (3): 468-473
 Sampson HA, Metcalfe DD. Food allergies. JAMA, 1992; 268 (20): 2840-2844.
 El Rafei A. et al. Diagnostic value of IgG4 measurement in patients with food allergy. Ann. Allergy, 1989; 62: 94-99.