



User's Manual

Transglutaminase IgA ELISA Kit



DEIA1832



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Anti-Tissue-Transglutaminase IgA is an indirect solid phase enzyme immunoassay (ELISA) for measurement of IgA class autoantibodies to tissue transglutaminase (tTG) in human serum or plasma.

Principles of Testing

Human recombinant tissue transglutaminase is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgA immunologically detects the bound patient antibodies forming a conjugate/anti-body/ antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyses to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgA antibodies present in the original sample.

Reagents And Materials Provided

Package size 96 determ.	
Qty.1	Divisible microplate consisting of 12 modules of 8 wells each, coated with recombinant tissue Transglutaminase IgA. Ready to use.
6 vials, 1.5 ml each	Combined calibrators with IgA class Anti-tTG antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaN ₃ <0.1% (w/w)) containing: IgA: 0; 5; 10; 25; 75; and 200 U/ml. Ready to use.
2 vials, 1.5 ml each	Anti-tTG Controls in a serum/buffer matrix (PBS, BSA, NaN ₃ <0.1%(w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed QC insert. Ready to use.
1 vial, 20 ml	Sample buffer (Tris, NaN ₃ <0.1% (w/w)), yellow, concentrate (5×).
1 vial, 15 ml	Enzyme conjugate solution (PBS, Proclin 300<0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgA; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution . Ready to use.
1 vial, 15 ml	Stop solution (contains acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, NaN ₃ <0.1% (w/w)), concentrate (50×).

Materials Required But Not Supplied

1. Microplate reader capable of endpoint measurements at 450 nm
2. Multi-Channel Dispenser or repeatable pipet for 100 µl
3. Vortex mixer
4. Pipets for 10 µl, 100 µl and 1000 µl
5. Laboratory timing device
6. Data reduction software
7. Distilled or deionized water
8. Graduated cylinder for 100 and 1000 ml

9. Plastic container for storage of the wash solution

Storage

1. Store the kit at 2-8°C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8°C.

Specimen Collection And Preparation

1. Collect whole blood specimens using acceptable techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

Reagent Preparation

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5×) with distilled or deionized water to a final volume of 100 ml prior to use.

Store refrigerated: stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50×) with distilled or deionized water to a final volume of 1000 ml prior to use.

Store refrigerated: stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples 1:100 with sample buffer before assay.

Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.

Controls are ready to use and need not be diluted.

Assay Procedure

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted samples.
2. Pipet 100 µl of calibrators, controls and prediluted patient samples in duplicate into the wells.

	1	2	3	4	5	6
A	SA	SE	P1	P5		
B	SA	SE	P1	P5		
C	SB	SF	P2	P..		
D	SB	SF	P2	P..		
E	SC	C1	P3			
F	SC	C1	P3			
G	SD	C2	P4			
H	SD	C2	P4			

SA-SF: standards A to F
P1, P2...C: patient sample 1, 2 ...
C1: positive control
C2: negative control

3. Incubate for 30 minutes at room temperature (20-28°C).
4. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
5. Dispense 100 µl of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
8. Dispense 100 µl of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add 100 µl of stop solution to each well of the modules and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with reference at 600-690 nm is recommended.

Note: The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Precautions

1. This kit is intended for Research Use Only. Not for use in diagnostic procedures.
2. Do not interchange kit components from different lots. Do not use kit components beyond their expiration dates.
3. Have all reagents and samples ready before start of the assay. All materials must be at room temperature (20-28°C). Once started, the test must be performed without interruption to get the most reliable and consistent results.
4. Perform the assay steps only in the order indicated.
5. Always use fresh sample dilutions.
6. Pipette all reagents and samples into the bottom of the wells.
7. To avoid carryover contaminations change the tip between samples and different kit controls.
8. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
9. All incubation steps must be accurately timed.
10. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.



11. Do not re-use microplate wells.
12. For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated.
13. Components containing human serum were tested and found negative for HBsAg, HCV, HIV 1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV 1 and HIV2 and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
14. Avoid contact with the TMB (3,3',5,5'-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
15. Avoid contact with the Stop Solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
16. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 18. 19. 20.).
17. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
18. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
19. Do not pipette by mouth.
20. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
21. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

