CELIAC Microlisa

Microwell ELISA Test for the Qualitative Detection of celiac disease associated IgA Antibodies to tissue transglutaminase in Human Serum/ Plasma

1. SUMMARY AND EXPLANATION OF THE TEST

Celiac disease (CD) is an auto immune disease which occurs in predisposed individuals as a reaction to glutein sensitivity. After absorption in the lamina propria of the intestinal mucosa gliadin is deamidated by the tissue transglutaminase (tTG). Gluten is found in various cereals (Wheat, Barley, Rye). If patient with celiac disease consume food containing gluten, this will finally leads to damage to the mucous membranes of the small intestine. The corresponding IgA antibodies are closely corelated to the damage of the small intestine. The test is simple and can be rapidly performed and permit a qualitative assessment of concentration of the transglutaminase antibodies. Although the disease start as in tolerance to gliadins, antibodies to tissue transglutaminase (tTG) in the gut epithelium are characteristic of the disease whereas serum IgA against (tTG) are highly specific for celiac disease, antibodies to gliadin are less informative as they can also be detected in other enteropathy and even in healthy individuals.

2. INTENDED USE

Celiac Microlisa is an in-vitro qualitative enzyme immunoassay for the detection of anti tTG IgA antibodies in Human Serum or Plasma. The assay is intended to be used as an aid in the recognition and diagnosis of Celiac disease.

3. PRINCIPLE OF THE TEST

Celiac Microlisa test is an enzyme immunoassay based on indirect ELISA.
Activated tissue transglutaminase (human recombinant) immuno-dominant epitopes are coated on the microtiter wells. Specimens and controls are added to the microtiter wells and incubated. Anti-transglutaminase antibodies if present in the specimen, will bind to the specific antigen absorbed on the surface of the wells. The plate is then washed to remove the unbound material. Horseradish proxidase (HRP) conjugated anti-human IgA added to each wells. This conjugate will bind to tTG-Ag-Ab complex present. Finally substrate solution containing chromogen and hydrogen proxide is added to the wells and incubated. A blue colour will develop in proportion to the amount of anti-tranglutaminase antibodies if present in the specimen. The colour reaction is stopped by a stop solution. The enzyme substrate reaction is read by EIA reader for absorbance at wavelength of 450 & 630 nm. If sample does not contain anti-transglutaminase antibody, then enzyme conjugate will not bind and the solution in the well will be either colourless or only a faint background colour develops.

4. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2016.


Manufactured By


No. of tests

Lot Number
Batch Number


Manufacturing Date


Expiry Date


Do not use if package is damaged


Keep Dry

In vitro diagnostic medical device


See Instruction for use

Temperature Limitation


Caution
See instruction for use

Catalogue Number


Keep away from sunlight

5. PACK SIZE

- 96 Tests

6. COMPONENTS IN EACH CELIAC MICROLISA KIT

Store all components at 2-8°C when not in use. Expiry date on the kit indicates that beyond which the kit should not be used.

COMPONENT	DESCRIPTION	96 TESTS
Microwells	Breakway microwells coated with activated tTG recombinant proteins packed in a pouch with dessicant.	12 Strips (96 wells)
Sample Diluent	Buffer containing protein stabilizers and antimicrobial agents as preservative.	2 Bottle (50 ml.)
Enzyme Conjugate (Ready to use)	Anti-human IgA conjugated with horseradish peroxidase with protein stabilizers.	1 Bottle (12 ml.)
Wash Buffer Concentrate (25x)	PBS with surfactant. Dilute 1:25 with distilled water before use.	1 Bottle (50 ml.)
TMB Substrate	To be diluted with TMB diluent before use.	1 Bottle (10 ml.)
TMB Diluent	Buffer solution containing H_2O_2 with preservative	1 Bottle (10 ml.)
Calibrator-1	2 RU/ml (IgA, human) (RTU)	1 Vial (1.5 ml.)
Calibrator-2	20 RU/ml (IgA, human) (RTU)	1 Vial (1.5 ml.)
Calibrator-3	200 RU/ml (IgA, human) (RTU)	1 Vial (1.5 ml.)
Control — ⚠	Normal human serum negative for Celiac antibodies. (RTU)	1 Vial (1.5 ml.)
Control + ⚠	Inactivated and diluted human serum; positive for Celiac antibodies and non-reactive for HIV, HBsAg and HCV, contains sodium azide as preservative.	1 Vial (1.5 ml.)
Stop Solution	Ready to use, 1N sulfuric acid.	1 Bottle (15 ml.)
Microwell Frame		1 No.
Plate Sealers	Adhesive backed sheets for sealing microwell plate/strips.	

7. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- Micropipettes and microtips.
- Elisa reader
- Distilled or deionized water
- Graduated Cylinders, for reagent dilution
- Disinfectant solution
- Paper towels or absorbent tissue
- Timer
- Elisa washer
- Incubator 37°C
- Vortex Mixer
- Disposable gloves
- Glassware

8. SPECIMEN COLLECTION & PREPARATION

1. Only human serum or plasma samples should be used for the test. While preparing serum samples, remove the serum form the clot as soon as possible to avoid hemolysis. Fresh serum/plasma samples are preferred.
2. Specimens should be free of microbial contamination and may be stored at 2-8°C for one week, or frozen at -20°C or lower. Avoid repeated freezing and thawing.
3. Use of heat inactivated, icteric hyperlipemic and hemolyzed samples. Moreover, Icteric hyperlipemic samples should be avoided as they may give erroneous results.

9. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

Celiac Microlisa test is best used with fresh samples that have not been frozen and thawed. However, most frozen samples will perform well if the procedure suggested below is followed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. If a centrifuge is available, the sample should be centrifuged. (10,000 rpm for 15 min.)

(B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of etiologic agents.

11. WARNING & PRECAUTION

⚠ **CAUTION:** THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. Controls, calibrators and Sample diluent contain Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976.)
10. Stop solution contains sulfuric acid. If sulfuric acid comes in contact with the skin, wash thoroughly with water. In case of contact with eyes, flush with excess of water.
11. ELISA Reader & micropipettes used in testing should be calibrated at regular interval to ensure accurate results.

12. PRECAUTIONS FOR USE

Optimal assay performance requires strict adherence to the assay procedure described in the manual.

1. Do not use kit components beyond the expiration date, which is printed on the kit.
2. Avoid microbial contamination of reagents. The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
3. Stop solution contains sulfuric acid. If sulfuric acid comes in contact with the skin, wash thoroughly with water. In case of contact with eyes, flush with excess of water.
4. Take care while preparing working substrate solution as vials of TMB Substrate & TMB Diluent are of same size.
5. Prepare working substrate solution just 10 minutes prior to adding in the wells.
6. If blue colour or white particles appears in working substrate solution then do not use it. Take fresh containers and tips and prepare it again.
7. Use separate tips for TMB substrate and TMB diluent.
8. Do not allow microwells to dry once the assay has started.
9. Ensure that the microwell strips are levelled in the strip holder. Before reading, wipe the bottom of the microwell strips carefully with soft, absorbent tissue to remove any moisture.
10. If available, a microwell reader which contains a reference filter with settings at 620 or 630 nm should be used. Use of a reference filter minimises interference due to microwells that are opaque, scratched or irregular. However, if a reference filter is unavailable, the absorbance may be read at 450 nm without a reference filter.
11. Distilled or deionised water must be used for wash buffer preparation.
12. Bring all the reagents to room temperature (20-30°C) before use.
13. Do not combine reagents from different batches, as they are optimized for individual batch to give best results.
14. Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid contamination of any sort.
15. Run calibrator, negative and positive controls in each assay.
16. Use freshly collected, clean serum samples for assay. Try to avoid Haemolyzed turbid, lipemic serum or plasma samples.
17. Use a separate tip for each sample and then discard it as biohazardous waste.
18. Thorough washing of the wells is critical to the performance of the assay.
19. Avoid strong light exposure during the assay.

13. PREPARATION OF REAGENTS

Prepare the following reagents before or during assay procedures. Reagents and samples should be at room temperature (20-30°C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8°C after use. All containers used for preparation of reagents must be cleaned thoroughly and rinsed with distilled or deionized water. Prewarm the incubator at 37°C.

1. Celiac Microlisa Strip:
Bring foil pack to room temperature (20-30°C) before opening to prevent condensation on the microwell strips.

a. Break-off the required number of strips needed for the assay and place in the well holder. Take the strip holder with the required number of strips, taking into account that one each of negative control, positive control and Calibrator-1, Calibrator-2 and Calibrator-3 should be included in each run.

b. Unused wells should be stored at 2-8°C, with dessicant in an aluminium pouch with clamp & rod. Microwells are stable for 30 days at 2-8°C from the date of opening of sealed pouch, when stored with desicant along with clamp & rod.

Caution: Handle microwell strip with care. Do not touch the bottom exterior surface of the wells.

2. Sample Preparation:
TUBE DILUTION : Mark the tubes carefully for the proper identification of the samples. Dilute the serum samples to be tested, with sample diluent 1:200 in separate tubes (1ml diluent + 5 µl samples). Use a separate tip for each sample and then discard as biohazardous waste. Ensure thorough mixing of samples to be tested.

3. Preparation of Wash Buffer:

a) Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37°C until all crystals dissolve.

b) Prepare at least 50 ml. (2ml. concentrated buffer with 48 ml. water) of buffer for each microlisa strip used. Mix well before use.

c) Mix 20 ml. 25 X wash buffer concentrate with 480 ml. of distilled or deionized water. Wash buffer is stable for 2 months when stored at 2-8°C.

4. Preparation of working substrate solution :
Mix TMB substrate and TMB Diluent in 1:1 ratio to prepare working substrate.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
TMB Substrate (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
TMB Diluent (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0

Do not store working substrate. Prepare a fresh dilution for each assay in a clean plastic/glass vessel. Determine the quantity of working substrate solution to be prepared from table. Mix solution thoroughly before use. Discard unused solution. A deep blue color present in the substrate solution indicates that the solution has been contaminated and must be discarded.

14. WASH PROCEDURE:

1. Incomplete washing will adversely affect the test outcome.
2. Aspirate the well contents completely into a waste container. Then fill the wells completely with wash buffer avoiding overflow of buffer from one well to another and allow to soak (approx. 30 seconds). Aspirate completely and repeat the wash and soak procedure 4 additional times for a total of 5 washes.
3. Automated washer if used should be well adjusted to fill each well completely without over filling
4. Tap upside down on absorbent sheet till no droplets appear on the sheet, taking care not to dislodge the wells.

15. TEST PROCEDURE

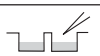
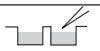
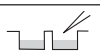
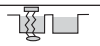
Note: *Once the assay has started, complete the procedure without interruption. All the reagents should be dispensed in the centre of the well and the tip of the pipette should not touch the wall of the microwell.*

Fit the stripholder with the required number of Celiac Microlisa strips. **The instructions of the procedure must be strictly followed.** Arrange all controls in a horizontal or vertical configuration. Configuration is dependent upon ELISA reader software.

1. Add 100 µl Negative Control in A-1 well.
2. Add 100 µl Calibrator-1 in C-1, Calibrator-2 in D-1 & Calibrator-3 in E-1 wells.
3. Add 100 µl Positive Control in B-1 well.
4. Add 100 µl diluted sample, in each well starting from F-1 well. (***Refer Sample Preparation : Point 13***(2).

5. Apply cover seal.
6. Incubate at 37°C ± 2°C for 30 min. ± 2 min.
7. Take out the plate form the incubator after the incubation time is over and, wash the wells 5 times with Working Wash solution according to the wash procedure given in the previous section (wash procedure).
8. Add 100 µl of Enzyme Conjugate Solution in each well.
9. Apply cover seal.
10. Incubate at 37°C ± 2°C for 30 min. ± 2 min.
11. Aspirate and wash as described in step no. 8.
12. Add 100 µl of working substrate solution in each well.
13. Incubate at room temperature (20 - 30°C) for 30 min. in dark.
14. Add 100 µl of stop solution.
15. Read absorbance at 450 nm within 30 minutes in ELISA READER. (Bichromatic absorbance measurement with a reference wavelength 600 - 650 nm is recommended when available).

16. SUMMARY OF PROCEDURE

Dilute Sample		Sample 5 µl — Sample Diluent 1 ml
Add Calibrator* Controls* & Sample		100 µl
Cover the plate & incubate		30 mins. at 37°C
Wash		5 Cycles
Add Enzyme Conjugate*		100 µl
Cover the plate & incubate		30 mins. at 37°C
Wash		5 Cycles
Prepare Chromogenic Substrate		No of Strips: 1 2 3 4 5 6 7 8 9 10 11 12 TMB Substrate (ml): 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 TMB Diluent (ml.): 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0
Add Substrate		100 µl
Incubate in dark		30 mins. at Room Temp.
Add Stop Solution		100 µl
Read Results		450 nm./630 nm.

**** Controls, Calibrators and Enzyme conjugate are ready to use & hence no dilution required.***

17. CALCULATION OF RESULTS

Ensure the following is within specified acceptance criteria

ii) NC O.D. must be ≤ 0.150. If it is not so, the run is invalid and must be repeated.

ii) Calibrator-2 O.D. should be more than 0.300. If it is not so, the run is invalid and must be repeated.

18. CALCULATION OF RESULTS

A) Semi-Quantitative

a. Cut off value = O.D. of Calibrator-2

b. Calculation of sample O.D. ratio : Calculate sample O.D. ratio as follows:

$$\text{Sample O.D. ratio} = \frac{\text{Sample O.D.}}{\text{Cut off Value}}$$

B) Quantitative

The standard curve from which the concentration of antibodies in the serum samples can be taken is obtained by point-to-point plotting of the obsorbance value measured for the 3 calibrators against the corresponding units (linear/linear)

19. INTERPRETATION OF RESULTS

A) Semi-Quantitative

(i) If the sample O.D. ratio is < 1.0, then interpret the sample as Negative for Celiac IgA antibodies.

(ii) If the sample O.D. ratio is > 1.0, then interpret the sample as Positive for Celiac IgA antibodies.

B) Quantitative

(i) If the Celiac IgA Units is < 20 RU/ml then interpret the sample as Negative for Celiac IgA antibodies.

(ii) If the Celiac IgA Units is > 20 RU/ml then interpret the sample as Positive for Celiac IgA antibodies.

(iii) If the absorbance of serum sample is above the value of calibrator-3 (200 RU/ml) the result should be read as >200 RU/ml. It is recommended that the sample be re-tested at a dilution of 1:800. the result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

20. LIMITATIONS OF THE ASSAY

1. The Celiac Microlisa is for *in vitro* diagnostic use only.
2. The test should be used for the detection of celiac Antibody in serum or plasma only and not in other body fluids.
3. **This is only a Screening test.** All positive samples should be confirmed by running test on another ELISA kit. Therefore for a definitive diagnosis, the patient's clinical history, symptomatology as well as serological data, should be considered. The results should be reported only after complying with above procedure.
4. Additional follow up testing using available clinical methods (along with repeat Celiac Microlisa test) is required, if Celiac Microlisa test is non-reactive with persisting clinical symptoms.

21. PERFORMANCE CHARACTERISTICS

The performance of Celiac Microlisa has been evaluated in-house with the panel of 1119 clinical serum/ plasma samples. The sensitivity was checked with 125 positive samples of patients with disease related to Celiac. The specificity is checked with a panel of 982 negative samples and 12 cross-reacting samples with other diseases; HBsAg, HIV, HCV, RA, CRP, ASO. The results obtained are as follows:

No. of Samples	Status	Celiac Microlisa	
		Positive	Negative
125	Celiac Positive	125	0
982	Celiac Negative	0	982
12	Cross-reacting	0	12

SENSITIVITY: 100% **SPECIFICITY: 100%**

Precision : Within-run and between-run precisions have been determined by testing 10 replicates of seven specimens : two negative, five celiac antibody positive (a strong, two medium and two weak positive). The C.V.(%) of negative and positive sample values were within 10%.

22. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in-vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

23. REFERENCES

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2. Tursu A, Brandimarte G, Giorgetti G M. Prevalence of anti-tissue transglutaminase antibodies in different degrees of intestinal damage in celiac disease. J Clin. Gastroenterol 36 (2003).
3. Ossendrof M. Schlumberger W, Schnider T, Orth T, Stocker W, Prellwitz W. Diagnostic relevance of antibodies against tissue transglutaminase, endomys, reticulin and gliadin in celiac disease. In corad K, Humbel R-L, Neurer M, Shoenfeld, Y, Pathogenic and diagnostic relevance of auto antibodies (1998).
4. Wahab PJ, Meijer JW, Dumitra D, Goeres MS, Mulder CJ, Celiac disease: more than willous atrophy, ROM J Gastoenterol 11 (2002).

24. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. No colour developed at the end of assay	a) Any one reagent has been added in wrong sequence.	Follow the procedure meticulously & repeat assay.
	b) Inactivated conjugate, improper storage	Check storage of enzyme conjugate and it should be free of any contamination.
	c) Microplate inactivated, due to improper storage	Keep unused strips in aluminium poly pouch with the dessicant pouch inside and proerly closed with clamp & rod.
	d) Inactivated substrate, improper storage or preparation	Use freshly prepared substrate solution. Recheck procedure, repeat assay
	e) Omission of any step in test procedure	Follow the procedure meticulously & repeat assay.
	f) Incorrect temperature timing or pipetting	Check procedure & repeat assay
	g) Improper preparation of reagents, error of dilution, improper mixing of reagents.	Check procedure & repeat assay
2. High O.D. value of Negative control	a) Plate not stopped after 30 minutes of additing stop solution	Follow the procedure meticulously & repeat assay.
	b) Sample microtip used for Positive and negative controls	Change micropipette tips while addition of negative/ positive control
	c) Nonspecific attachment/ binding of other reagent	If plates get scratches/ aberrations during washing, non specifi proteins may bind while addition of next step.
3. Too much colour in all wells of the plate (high background)	a) Contaminated substrate	Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container.
	b) Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer conc.	Check the container and quality of water used for dilution. Use of glass distilled water is preferred.
	c) Over incubation of substrate and delay in addition of stop solution.	Follow the procedure meticulously.
	d) Insufficient washing. i) Washing not consistent ii) Filling volume not sufficient. iii) Insufficient no. of wash cycles. iv) Contaminated wash device	Check wash device, fill the well close to the top. After washing, blot the microwells on absorbent tissue. Follow wash protocol meticulously
	e) Use of wash solution from other manufacturer.	Use only Covid Kawach IgG Microlisa wash solution.
	f) Working substrate not protected from light	Incubate the plate in dark after addition of substrate.
4. Poor reproducibility	a) Washing problems.	Check all 8 ports/ manifold for uniform flow of wash buffer. If there are blockage, clen the ports.
	b) Uncalibrated pipettes or tips not well fitted, improper pipetting/ dispensing.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	c) Interference in optical pathway due to Air bubbles.	Clean or dry the bottom of microwells, check for bubbles and repeat the readings.
5. False Positive	Beside 3a, b, c, d, e & f incorrect interpretation and calculation of final results	Check the calculation part given in the insert and correctly interpret.
6. False Negative/ low O.D. of Positive control & positive sample	a) Inadequate addition of substrate/conjugate solution.	Follow the procedure meticulously & repeat assay.
	b) Kit expired, reagent of different kit used.	Check the expiry of the kit before use.
	c) White particles in working substrate solution.	Discard the substrate and prepare the working substrate again in fresh tube.
	d) Uncalibrated pipettes, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	e) Deterioration of Enzyme conjugate	Check storage of Enzyme conjugate. It shall be stored at 2-8°C.
	f) Stop solution is added before 30 minutes. Reaction terminated before 30 minutes.	Follow the test procedure meticulously.
	g) O.D. taken at incorrect wavelength.	Read O.D. values at 450 nm and 630 nm.

in vitro diagnostic Reagent, not for medicinal use

VER-01 R-02

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