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 British and European Standard EN ISO 15223-1:2016.

	Manufactured By	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution See instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		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 Concentrate (50x)	Anti-human IgA conjugated with horseradish peroxidase with protein stabilizers.	1 Vial (0.4 ml.)
Conjugate Diluent	Buffer containing protein stabilizers.	1 Bottle (15 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
- Sodium hypochlorite 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 and Icteric hyperlipemic samples should be avoided as 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.

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 Enzyme Conjugate:
Dilute conjugate concentrate 1:50 in conjugate diluent. **Do not store working conjugate**. Prepare a fresh dilution for each assay in a clean glass vessel. Determine the quantity of working conjugate solution to be prepared from table given below. Mix solution thoroughly before use.

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
Enzyme Conjugate Concentrate (µl)	20	40	60	80	100	120	140	160	180	200	220	240
Conjugate Diluent in (ml)	1	2	3	4	5	6	7	8	9	10	11	12

Note: In case any precipitate is found in conjugate diluent/sample diluent, it should be allowed to settle and the supernatant can be used for the test. The precipitate does not interfere with the working of the kit.

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

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.

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 Susbstrate (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

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. While the samples are incubating, prepare Working Wash Solution and Working Conjugate as specified in Preparation of Reagents.
8. 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).
9. Add 100 µl of Working Conjugate Solution in each well.
10. Apply cover seal.
11. Incubate at 37°C ± 2°C for 30 min. ± 2 min.
12. Aspirate and wash as described in step no. 8.
13. Add 100 µl of working substrate solution in each well.
14. Incubate at room temperature (20 - 30°C) for 30 min. in dark.
15. Add 100 µl of stop solution.
16. 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
Prepare working conjugate		No of Strips: 1 2 3 4 5 6 7 8 9 10 11 12 Enz. conc. (µl): 20 40 60 80 100 120 140 160 180 200 220 240 Diluent (ml.): 1 2 3 4 5 6 7 8 9 10 11 12
Add 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 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 fresh as well as frozen samples. The testing has been done with clinical samples, samples from random blood donors, cross reacting samples; RA, CRP, ASO and patients with diseases related to Celiac. The results of in-hoouse studies are as follows:

No. of Samples	Status	Celiac Microlisa + ve	Celiac Microlisa - ve
125	All ELISA +ve	125	-
994	All EIA -ve	-	994

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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24. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Control out of validation limit	a) Incorrect temperature timing or pipetting	Check procedure & repeat assay
	b) Improper preparation of reagents, error of dilution, improper mixing of reagents.	Check procedure & repeat assay
	c) Cross contamination	Pipette carefully and do not interchange caps. Repeat assay
	d) Incorrect reading filter or readings without blanking the reader.	Check the filter used. It should be 450nm. If no reference filter is used absorbance will increase.
	e) Interference in the optical pathway	Check the reader. Clean or dry the bottom of micro wells, check for bubbles & repeat the readings.
	f) Used components from different lots.	Do not use components from different lots as they are adjusted for each batch released.
	g) Expired Reagents	Check the kit expiry date. Use the kit with-in shelf life
2. No colour or light colour developed at the end of assay	a) Any one reagent has been added in wrong sequence.	Check procedure and repeat assay.
	b) Inactivated conjugate, wrong dilution used, improper conservation	Check for contamination, recheck procedure
	c) Microplate inactivated, due to improper conservation	Keep unused strips in sealable plastic bag, very well closed with the dessicant pouch inside
	d) Inactivated substrate, improper conservation or preparation	Use freshly prepared substrate solution Recheck procedure, repeat assay
3. Too much colour in all wells of the plate	a) Contaminated substrate use of same container for preparing & dispensing substrate & conjugate.	Check TMB Diluent it should be colourless. If blue in colour then discard and use acid washed or disposable container.
	b) Contaminated or improper dilution of reagents.	Check for contamination, check dilutions.
	c) Contaminated washing solution (1X).	Check the container and quality of water used for dilution.
	d) Over incubation of substrate and delay in addition of stop solution.	Repeat assay.
	e) Insufficient washing.	Check wash device, fill the
	i) Washing not consistent	well close to the top.
	ii) Filling volume not sufficient.	After washing, blot the
	iii) Insufficient no. of wash cycles.	microwells on absorbent tissue.
	iv) Contaminated wash device	
	f) Use of wash solution from other manufacturer.	Use only Celiac Microlisa wash solution.
4. Poor reproducibility	a) Washing problems.	
	b) Uncalibrated pipettes or tips not well fitted, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	c) Reagent & sera not at room temperature or not well mixed before use.	Equilibrate reagents to room temperature and mix thoroughly before use
	d) Too long time for addition of samples or reagents, Inconsistency in time intervals	Develop consistent and uniform technique.
	e) Interference in optical pathway due to Air bubbles.	1e.
5. False Positive	Beside 3a, b, c, d, e incorrect interpretation and calculation of final results	Check the calculation part given in the insert and correctly interpret.
6. False Negative/ low O.D. for PC and positive sample	a) Inadequate addition of substrate/conjugate solution	Recheck the test procedure and reagent volume.
	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.

For *in vitro* diagnostic use only, not for medicinal use

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