Instruction

WIESLAB® Celiac hs Screen

Enzyme immunoassay for detection of autoantibodies against celiac disease specific peptide antigen

Micro titration 96 wells
Store the kit at +2-8° C

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For Research Use Only. Not for use in diagnostic procedures.

REF **CELI SC RUO**

Σ 96

PURPOSE OF RESEARCH PRODUCT

The Wieslab® Celiac hs Screen kit is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of IgG antibodies in human serum to celiac disease (CD) specific peptide antigen. The assay is used to detect antibodies in a single serum specimen.

The result shall not be used for clinical diagnosis or patient management.

Summary and explanation

Celiac disease is caused by intolerance to gluten, a complex mixture of proteins found in wheat, barley and rye. Once the disease has developed it can only be treated by the introduction of a lifelong gluten-free diet (1,2,3).
It is generally accepted that the prevalence of celiac disease is 0.5 to 1% in the Western Hemisphere and a similar occurrence is likely in North Africa, the Middle East, India and parts of China. This makes celiac disease one of the most prevalent food induced disorders known (4,5,6,7). Most of those patients, however, are not diagnosed due to the diversity of symptoms associated with celiac disease, general lack of knowledge about celiac disease among general practitioners and gastroenterologists, and the invasive endoscopic procedure that is still regarded necessary for a proper diagnosis (8).
It is now well established that celiac disease is caused by aberrant T-cell mediated immune responses to the gluten proteins (9). This inflammatory T- cell response leads to the tissue destruction and remodelling that is typically found in the upper small intestine of celiac disease patients and resolves on a gluten-free diet (10). Strikingly, such T-cell responses are largely directed against modified gluten proteins. The modification, the conversion of the amino acid glutamine into glutamic acid, is introduced due to the activity of the enzyme tissue transglutaminase 2 in the small intestine. As a by-product of the T-cell response to modified gluten, patients usually also produce antibodies to both tissue transglutaminase 2 and modified gluten. As these antibodies are secondary to the T-cell response to gluten, their presence is highly predictive for celiac disease (11,12).

Principle of the Wieslab® Celiac hs Screen

The wells of the microtiter plate are coated with purified synthetic peptide antigen. The antigen is a unique gluten derived, deamidated peptide, recognized by T-cells in the initial immune response of celiac disease.
During the first incubation, specific antibodies in diluted serum, will bind to the antigen coating.
The wells are then washed to remove unbound antibodies and other components.
A conjugate of alkaline phosphatase-labelled antibodies to human IgG binds to the antibodies in the wells in the second incubation.
After a further washing step, detection of specific antibodies is obtained by incubation with a substrate solution. The amount of bound antibodies correlates to the colour intensity and is measured in terms of absorbance (optical density (OD)). The absorbance is calculated against a calibrator curve and the results are given in arbitrary U/mL.

Warnings and precautions

- For research use only. Not for use in diagnostic procedures.
- The human serum components used in the preparation of the controls and calibrators in the kit have been tested for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen by FDA approved methods and found negative. Because no test methods can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
- The Centres for Disease Control and Prevention and National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
- All solutions contain ProClin 300 as a preservative. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing ProClin may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.
- The concentrations of antibodies in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

- Material safety data sheets for all hazardous components contained in this kit are available on request from Euro Diagnostica.

BUF	WASH	30X
DIL		
CONJ		
CAL		

CONTROL	+
CONTROL	-
SOLN	STOP
SUBS	pNPP

Warning

Contains ProClin 300:
Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC no. 220-239-6] (3:1)

H317:	May cause an allergic skin reaction.
P264:	Wash hands thoroughly after handling.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P302+352:	IF ON SKIN: Wash with plenty of soap and water.
P333+313:	If skin irritation or rash occurs: Get medical advice/attention.

Specimen collection

The Wieslab® Celiac hs Screen assay is recommended for serum samples. Handle as if capable of transmitting infectious agents.
Avoid using sera which are icteric, lipemic and haemolysed. Heat-inactivated sera can yield unspecific reactivity's and should not be used. Store serum between 2-8°C if testing will take place within five days. If specimens are to be kept for longer periods, store at -20°C or colder. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results. The CLSI provides recommendations for storing blood specimens, Approved Standard-Procedures for the Handling and Processing of Blood Specimens, NCCLS H18 A3.

Kit components and storage of reagents

- One frame with 96 wells coated with peptide, with lid, sealed in a foil pack with a dry pack
- 1.5 mL negative control (NC) containing human serum in diluent (green colour)
- 1.5 mL positive control (PC) containing human serum in diluent (red colour)
- 13 mL conjugate containing alkaline phosphatase labelled antibodies to human IgG (blue colour)
- 2 x 32 mL Diluent (Dil) containing PBS (red colour)
- 13 mL Substrate pNPP
- 13 mL Stop solution
- 30 mL Wash solution 30x concentrated
- Six calibrators (five calibrators, Cal 2-6, containing human serum) in diluent (red colour). 1.5 mL Cal 1 = 0 U/mL, 1.5 mL Cal 2 = 2 U/mL, 1.5 mL Cal 3 = 10 U/mL, 1.5 mL Cal 4 = 30 U/mL, 1.5mL Cal 5 = 100 U/mL, 1.5 mL Cal 6 = 200 U/mL.

All reagents in the kit are ready to use, except wash solution, and should be stored at 2-8°C. Remove only the number of wells needed for testing, reseal the aluminium package carefully.

Materials or equipment required but not provided

- Microplate reader with filter 405 nm
- Precision pipettes with disposable tips
- Washer for plates, absorbent tissue, tubes and timer

PROCEDURE
All solutions should be used at room temperature. Mix reagents by gentle inversion before use.
Incubate all steps at room temperature (20-28°C) with lid. Incubate serum for 60 minutes, conjugate for 30 minutes and substrate for 30 minutes.
Do not use components past the expiration date and do not not intermix components from different lots.
Optimal results will be obtained by strict adherence to this protocol.

Preparation of washing solution
Dilute 10 mL of 30x concentrated wash solution in 290 mL distilled water.
When stored at 2-8°C, the diluted wash solution is stable until the date of expiry of the kit.

Sample dilution and incubation
Dilute the patient's serum 1/100 with diluent (990 µL diluent + 10 µL serum) and mix well.
Pipette 100 µL/well in duplicate of Calibrator 1, 2, 3, 4, 5, 6, PC, NC and diluted patient's serum (P) according to the diagram below. Incubate for 60 minutes.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal 1	Cal 5	P1									
B	Cal 1	Cal 5	P1									
C	Cal 2	Cal 6	P2									
D	Cal 2	Cal 6	P2									
E	Cal 3	PC	etc.									
F	Cal 3	PC										
G	Cal 4	NC										
H	Cal 4	NC										

Please note: Rapid dispensing during the assay procedure is important to avoid assay drift. Dilution of samples in microtiter wells and use of 8-channel pipette for transfer of samples is recommended.

After sample incubation
Wash 3 times with 300 µL washing solution/well, filling and emptying the wells each time, after the last wash, empty the wells by tapping the strip on an absorbent tissue.

Adding conjugate
Add 100 µL conjugate to each well. Incubate for 30 minutes.

After conjugate incubation
Wash as before.

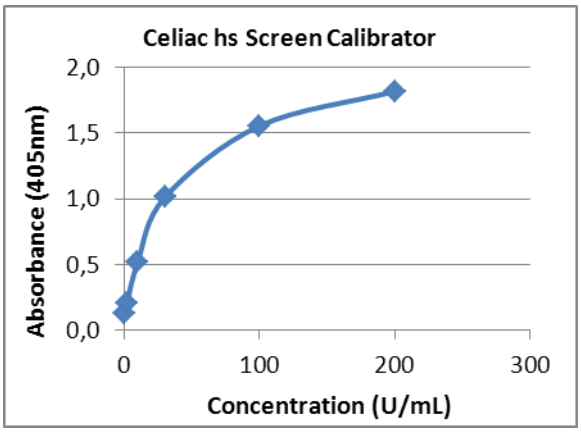
Adding substrate solution
Add 100 µL substrate pNPP to each well. Incubate for 30 minutes.

Adding stop solution
Add 100 µL stop solution to each well and read the absorbance at 405 nm on a microplate reader within 2 hours.

Calculations
Construct a calibrator curve by plotting the OD against the U/mL values of the 6 calibrators (table below) by using 4 parameter logistic curve fit. Read the U/mL value of the patient sample from the constructed curve. Values greater than 200 should be reported as >200, or reassayed with a higher dilution.

Example

Calibrator	U/mL	Absorbance
1	0	0.133
2	2	0.209
3	10	0.524
4	30	1.013
5	100	1.556
6	200	1.819



A sample with an absorbance value of 0.856 will read on the X-axis as having 22 U/mL.
In this example a 4 parameter logistic curve fit has been applied.

Important: The curve is an example and should not be used for actual patient interpretation.

Quality Control
The OD for calibrator 1 should be < 0.2.
The OD for calibrator 6 should be > 1.0.
The value for the positive and negative controls, see lot certificate.
The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cut-off. It is recommended to assay an additional control at the assay cut-off. As the positive control is ready-to-use it does not indicate an eventual dilution error by the user. It is recommended to use an internal control for this purpose.
If any of the values are not within their respective ranges, the test should be considered invalid and the test should be repeated. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organisations. Refer to CLSI C24-A for guidance on appropriate QC practices.

Interpretation of results
≤ 3 U/mL = **Negative**
> 3 U/mL = **Positive**

The cut-off level was established on basis of results from 120 apparently healthy blood donors: 60 men and 60 female between 19-68 years have been analysed. No differences attributable to age or gender were observed in this population.
It is recommended that users establish reference ranges for the populations served by their own laboratories; the above is only intended as a guide to the interpretation of results.

Limitations
The individual samples antibody titre cannot be used as a measure of disease severity, as antibodies from different samples may differ from each other in affinity. Thus, it is difficult to obtain an absolute standardisation of results.
Anti-celiac antibodies may be seen in samples with no evidence of clinical disease. Also some samples with celiac disease may have undetectable antibodies. Anti-celiac antibody levels do not necessary correlate to disease state.

Results
The Wieslab® Celiac hs Screen kit measures antibodies against synthetic peptide antigen. The kit is calibrated in relative units using a positive patient serum pool. The six calibrators (1-6) have been assigned values of 0 U/mL (1), 2 U/mL (2), 10 U/mL (3), 30 U/mL (4), 100 U/mL (5) and 200 U/mL (6). These values have been chosen arbitrarily by Euro Diagnostica, since no generally recognised international standard exists for expressing the titre of antibodies to celiac disease specific peptide antigen.
The specificity and sensitivity were evaluated with 151 patients with diagnosed celiac disease, 80 non-celiac patients and 120 apparently healthy blood donors. The sensitivity was 87%. The specificity was 94% with non-celiac patients and 95% with apparently healthy blood donors.

Performance characteristics

Table 1. Clinical sensitivity and specificity. A total of 351 frozen retrospective sera with clinical characterisation were assayed. 151 samples were from celiac disease patients, 80 non-celiac disease samples and 120 samples from apparently healthy blood donors.
The following table summarises the results.

Control and Disease group	**Total number**	**Negative ≤3 U/mL**	**Positive >3 U/mL**
Blood donor samples	120	114	6
Non-celiac disease samples	80	75	5
Celiac disease samples	151	20	131

Clinical sensitivity
Celiac disease samples = 131/151 = 86.8% 95% CI = 81.3 - 92.2%

Clinical specificity
Non-celiac disease samples = 75/80 = 93.8% 95%CI = 86.0 - 97.9%
Blood donor samples = 114/120 = 95.0% 95%CI = 89.4 - 98.1%

The 95% confidence interval (CI) was calculated using the exact method.

Table 2. **Agreement between the Wieslab® Celiac hs Screen kit and another commonly used ELISA kit for detection of celiac disease specific antibodies.** A total of 231 frozen retrospective sera were assayed: 151 celiac disease samples and 80 non-celiac disease samples.

151 celiac disease samples (tTG IgA equivocal results included in positive)		
Assay	Wieslab® Celiac hs Screen	tTG-IgA ELISA
Cut-off	Negative?3 U/mL Positive>3 U/mL	Negative <7 U/mL Equivocal 7-10 U/mL Positive>10 U/mL
Positive	131	95
% positive	87%	63%

80 non-celiac disease samples (no tTG IgA equivocal results)		
Assay	Wieslab® Celiac hs Screen	tTG-IgA ELISA
Cut-off	Negative?3 U/mL Positive>3 U/mL	Negative <7 U/mL Equivocal 7-10 U/mL Positive>10 U/mL
Negative	75	79
% negative	94%	99%

Table 3. Batch to batch variation was determined by testing six different samples in duplicates on three different batches.

	1	2	3	4	5	6
Mean value (U/mL)	5	5	26	25	82	59
SD	0.91	0.69	1.81	2.37	14.28	5.55
% CV	20	14	7	10	18	9

Table 4. Inter-assay precision was determined by testing six different samples in eight replicates at three separate occasions.

	1	2	3	4	5	6
Mean value (U/mL)	4	12	35	25	84	139
SD	0.04	0.87	0.93	1.19	10.33	6.61
% CV	1	7	3	5	12	5

Table 5. Intra-assay precision was determined by testing six different samples in eight replicates at one occasion.

	1	2	3	4	5	6
Mean value (U/mL)	4	11	34	23	73	146
SD	0.26	0.62	1.33	0.71	4.54	4.37
% CV	6	6	4	3	6	3

Table 6. Dilution recovery was determined by testing five serial dilutions for three different samples.

Sample	**Dilution**	**Mean Measured Concentration (U/mL)**	**Calculated Concentration (U/mL)**	**Dilution Corrected % Recovery**
1	1/100	18.1	18.1	100
	1/200	8.6	9.1	95
	1/400	4.5	4.5	100
	1/800	2.4	2.3	104
	1/1600	1.3	1.1	118
Sample	**Dilution**	**Mean Measured Concentration (U/mL)**	**Calculated Concentration (U/mL)**	**Dilution corrected % Recovery**
2	1/100	31.6	31.6	100
	1/200	18.3	15.8	116
	1/400	9.1	7.9	115
	1/800	4.3	4.0	108
	1/1600	2.2	2.0	110
Sample	**Dilution**	**Mean Measured Concentration (U/mL)**	**Calculated Concentration (U/mL)**	**Dilution corrected % Recovery**
3	1/100	165.6	165.6	100
	1/200	99.1	82.8	120
	1/400	43.5	41.4	105
	1/800	21.3	20.7	103
	1/1600	9.5	10.4	91

Troubleshooting

Problem:	**Possible causes:**	**Corrective action:**
Control values out of range.	1. Incorrect temperature, timing or pipetting; reagents not mixed.	1. Check that the time and temperature was correct. See ”Poor precision” below. Repeat test.
	2. Cross contamination of controls.	2. Pipette carefully.
	3. Optical pathway not clean.	3. Check for dirt or air bubbles in the wells. Wipe bottom and reread.
All test results negative.	1. One or more reagents not added, or added in wrong sequence.	1. Recheck procedure. Check for unused reagent. Repeat test.
	2. Antigen coated plate inactive.	2. Check for obvious moisture in unused wells. Wipe bottom and reread.
All test results yellow.	1. Contaminated buffers or reagents.	1. Check all solutions for turbidity.
	2. Washing solution contaminated.	2. Use clean container. Check quality of water solution used to prepare.
	3. Improper dilution of serum.	3. Repeat test.
Poor precision.	1. Pipette delivery CV greater than 5%.	1. Check calibration of pipette. Use reproducible technique.
	2. Serum or reagents not mixed sufficiently or not equilibrated to room temperature.	2. Mix all reagents gently but thoroughly and equilibrate to room temperature.
	3. Reagent addition taking too long; inconsistency in timing intervals.	3. Develop consistent uniform technique and use multi-tip device or auto dispenser to decrease time.
	4. Optical pathway not clean.	4. Check for air bubbles in the wells. Wipe bottom and reread.
	5. Washing not consistent; trapped bubbles; washing solution left in the wells.	5. Check that all wells are filled and aspirated uniformly. Dispense liquid above level of reagent in well. After the last wash, empty the wells by tapping the plate on an absorbent tissue.
	6. Improper pipetting.	6. Avoid air bubbles in pipette tips.

References

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Explanation of symbols.

LOT	Batch number.
	Use by date.
	Temperature limit.
	Biological risks.
	Consult Instruction for use.
	Manufacturer.
	Warning.
 96	Contains sufficient for 96 tests.
REF	Catalogue number.

Ag	Antigen.
DIL	Diluent.
CONJ	Conjugate.
BUF **WASH** **30X**	Wash solution 30x conc.
SUBS **pNPP**	Substrate pNPP.
SOLN **STOP**	Stop solution.
CAL **X**	Calibrator.
CONTROL **X**	Control.

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