Version 24-04/24

User's Manual

Anti-Gliadin sIgA ELISA Kit

REF **DEIA9968**

Σ **96T**

RUO

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The assay is intended for the quantitative determination of anti-gliadin-sIgA antibodies in stool. For in vitro diagnostic use only.

General Description

The celiac/coeliac disease is caused by the gliadin fraction of wheat gluten and similar proteins of rye and barley. The disease is manly manifested as chronic digestion insufficiency in children or young adults. In addition, patients with celiac disease have a greatly increased risk of developing malignant T-cell lymphoma of the small bowel, as T-cell lymphoma was found in 6 of 10 patients with coeliac sprue. Early diagnosis of celiac disease is important, because there is evidence that a gluten-free diet might help to prevent complications and malignancies (including intestinal lymphoma).

The measurement of anti-gliadin-sIgA-antibodies may be useful as a screening criterion before jejunal biopsy, the "Gold Standard", and for the monitoring of the gluten-free diet treatment.

Indication:

1. Celiac disease
2. Food intolerance

Principles of Testing

The antigen gliadin is immobilized on the microtiter plate. During the first incubation step, the human anti-gliadin-sIgA antibodies in the samples are bound by the immobilized antigen. After a washing step, the anti-gliadin-sIgA antibodies of the samples are quantitatively determined by addition of a peroxidase-labeled anti-gliadin-sIgA antibody mix. Tetramethylbenzidine (TMB) is used as a peroxidase substrate. The enzymatic reaction is stopped by an acidic stop solution. The absorbance of the color compound is determined photometrically at 450 nm. The measured absorbance is directly proportional to the amount of bound anti-gliadin-sIgA antibodies.

Reagents And Materials Provided

1. One holder with precoated strips, 12 x 8 wells
2. ELISA wash buffer concentrate 10x, 2 x 100 ml
3. Conjugate (Peroxidase-labeled), ready to use, 1 x 15 ml
4. Control negative, lyophilized, 4 vials
5. Control positive, lyophilized, 4 vials
6. Cut-off Control, lyophilized, 4 vials
7. TMB substrate (Tetramethylbenzidine), ready-to-use, 1 x 15 ml
8. ELISA stop solution, ready to use, 1 x 15 ml
9. Extraction buffer concentrate, 2.5x, 1 x 100 ml

Materials Required But Not Supplied

1. Ultra pure water
2. Laboratory balance
3. Calibrated precision pipettors and 5-1000 µL tips
4. Foil to cover the microtiter plate
5. Horizontal microtiter plate shaker
6. Multi-channel pipets or repeater pipets
7. Vortex
8. Standard laboratory glass or plastic vials, cups, etc.
9. Microtiter plate reader

Storage

The reagents are stable up to the expiry date stated on the label when stored at 2-9°C. For more detailed information, please download the following document on our website.

Specimen Collection And Preparation

Sample storage

Raw stool:

Raw stool samples can be stored for 4 weeks at -20°C. Avoid repeated freezing and thawing.

Stool suspensions:

Stool extract can be stored for 7 days at 2-8°C or -20°C or for one day at room temperature (15-30°C). Avoid more than three freeze-thaw cycles.

1. Extraction of the stool samples:

Diluted extraction buffer is used as a sample extraction buffer. We recommend the following sample preparation: Stool Sample Application System (SAS).

Stool sample tube - Instructions for use.

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 1.5 ml extraction buffer:

Applied amount of stool: 15 mg

Buffer Volume: 1.5 ml

Dilution Factor: 1:100

Please follow the instructions for the preparation of stool samples using the SAS as follows:

a. The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.

b. Fill the empty sample tube with 1.5 ml of ready-to-use extraction buffer before using it with the sample. Important: Allow the extraction buffer to reach room temperature.

c. Unscrew the tube (yellow part of cap) to open. Insert yellow dipstick into sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.

d. Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with buffer for app. 10 minutes improves the result.

e. Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.

f. Carefully unscrew the complete cap of the tube including the turquoise ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution I: 1:100

2. Dilution of samples:

The suspension from the sample extraction (dilution I) ist further diluted 1:50 with wash buffer. For example:

20 µL dilution I + **980 µL** wash buffer, mix well = **1:50 (dilution II)**

This results in a final dilution of **1:5000**.

For analysis, pipet **100 µL** of **dilution II** per well.

Reagent Preparation

1. To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. Prepare only the appropriate amount necessary for each run. The kit can be used up to 4 times within the expiry date stated on the label.
2. Reagents with a volume less than 100 µL should be centrifuged before use to avoid loss of volume.
3. The ELISA wash buffer concentrate should be diluted 1:10 in ultra pure water before use (100 ml concentrate + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C before dilution of the buffer solutions. The buffer concentrate is stable at 2-8°C until the expiry date stated on the label. Diluted buffer solution (wash buffer) can be stored in a closed flask at 2-8°C for one month.
4. The extraction buffer concentrate should be diluted 1:2.5 in ultra pure water before use (100 ml concentrate + 150 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C before dilution of the buffer solutions. The buffer concentrate is stable at 2-8°C until the expiry date stated on the label. Diluted buffer solution (extraction buffer) can be stored in a closed flask at 2-8°C for three months.
5. The lyophilized controls, negative, positive and cut-off are stable at 2-8°C until the expiry date stated on the label. Reconstitution details are given in the data sheet. Diluted controls are not stable and can not be stored.
6. All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2-8°C.

Assay Procedure

Prior to use in the assay, allow **all reagents and samples** to come to **room temperature** (15-30°C) and mix well.

Take as many microtiter strips (PLATE) as needed from kit. Store unused strips covered at 2-8°C. Strips are stable until the expiry date stated on the label.

Wash the pre-coated microtiter plate **5 x with 250 µL ELISA wash buffer before use**. After the final washing step, the inverted microtiter plate should be tapped on absorbent paper.

Carry out the tests in duplicate.

1. Add 100 µl of controls, negative, positive and cut-off and diluted SAMPLE (patient samples).
2. Incubate for 1 hour at room temperature.
3. Decant the content of the plate and wash the wells 5 x with 250 µl ELISA wash buffer.
4. Add 100 µl of CONJ (conjugate).
5. Incubate for 1 hour at room temperature.
6. Decant the content of the plate and wash the wells 5 x with 250 µl ELISA wash buffer.
7. Add 100 µl SUB (TMB substrate).
8. Incubate for 10-20 minutes at room temperature*.
9. Add 100 µl STOP (ELISA stop solution) and mix shortly.
10. Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference.

* The intensity of the color change is temperature sensitive. We recommend observing the color change and stopping the reaction upon good differentiation.

Quality Control

CD recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

Based on an internal study with apparently healthy adults (n = 45) resulted in a reference value of < 100 U/l.

We recommend each laboratory to establish its own reference range.

Calculation

Samples with an optical density higher than the average optical density of the cut off control are positive. Cut off = ODcut off control = 100 U/l

Example:

ODpatient sample = 0.685

ODcut off control = 0.321 = 100 U/l

Concentration patient sample =(0.685*100 U/l)/0.321 = 213.4 U/l

Attention: Calculation is only valid for a sample dilution factor of 1:5000.

Reference Values

Based on an internal study with apparently healthy adults (n = 45) resulted in a reference value of < 100 U/l. We recommend each laboratory to establish its own reference range.

Sensitivity

36.2 U/L

Precautions

1. Control samples should be analyzed with each run.
2. Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
3. Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
4. The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

Limitations

The lower limit of the measurement range can be calculated as:

LoB × sample dilution factor to be used

Samples with concentrations lower than the measurement range cannot be clearly quantified.