Version 08-03/25

User's Manual

Milk Intolerance IgG kit

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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 Milk Intolerance IgG is an immunodot kit intended for the detection, in human sera only, of IgG autoantibodies against β-Lactoglobulin (cow's milk) and Soya antigens.

Antigens used

β-Lactoglobulin Cow milk protein (purified from cow milk)

Soya Soy proteins (purified from soybean flour)

Principles of Testing

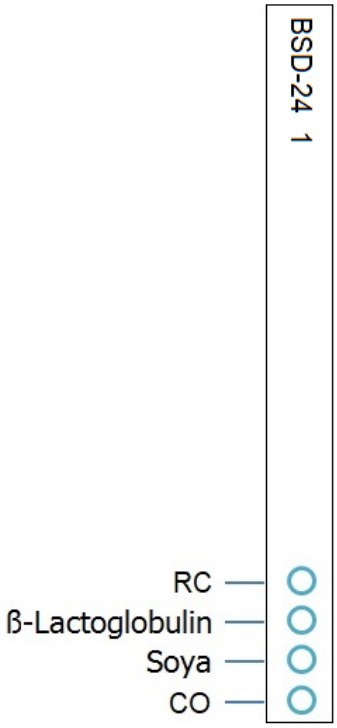
This kit and all its components are intended to be performed exclusively manually. The test is based on the principle of an Enzyme Immunoassay. The strips are composed of a membrane fixed on a specific plastic support. During the test procedure, the strips are incubated with diluted patients' sera. Human antibodies, if present, bind to the corresponding specific antigen(s) on the membrane. Unbound or excess antibodies are removed by washing. AP-conjugated goat antibodies against human IgG are added to the strips. This enzyme conjugate binds to the antigen-antibody complexes. After removal of excess conjugate by washing, a substrate solution is added. Enzyme activity, if present, leads to the development of purple dots on the membrane pads. The intensity of the coloration is directly proportional to the amount of antibody present in the sample.

The kit is composed of 24 single-use tests.

Reagents And Materials Provided

Prior to any use of the kit, please check that all the items listed are present or if characteristics of the product are not corresponding to those described hereafter.

1. (10×) Wash buffer, 1×40 ml, 10× concentrated, Contains: H_2O • TBS • NaCl • Tween • Preservatives
2. Dot strips, 24 units, 4 dots each: 1 negative control (CO), 2 antigens, 1 positive control (RC)
3. Diluent buffer, 1×40 ml, Contains: H_2O • TBS • NaCl • Tween • BSA • Preservatives • Dye
4. Conjugate, 1×40 ml, Contains: H_2O • TBS • NaCl • KCl • MgCL2 • AP-conjugated goat anti-human IgG • Preservatives • Dye
5. Substrate, 1×40 ml (brown bottle, pale yellow solution), Contains: H_2O • Preservatives • MgCL2 • TBS • NBT • BCIP • NBT Stabilizer



Materials Required But Not Supplied

Platform shaker / Micropipettes / Timer / Graduated cylinder / Distilled or deionised water / Tweezers / Absorbent and/or filter paper.

Storage

The reconstituted Wash Solution is stable for at least one month at 2-8°C. Reagents and strips can be stored at 2-8°C until the expiry date indicated on each vial or tube.

Place unused strips back into the provided tube, seal it and store at 2-8°C. Chromogen/Substrate (NBT/BCIP) shall be stored at 2-8°C.

When stored properly, all test kit components are stable until the indicated expiry date.

Specimen Collection And Preparation

The test should be used on recently collected sera samples only! Sera with particles should be centrifuged at low speed. Blood samples should be collected in dry tubes or tubes containing EDTA or heparin. Please avoid using a pool of different sera, as this can lead to inconsistent results. After separation, the serum samples should be used immediately or aliquoted and stored at 2-8 ° C (for storage for a few days) or frozen at -20°C (for longer storage periods). Repeated freezing/ thawing cycles of the samples must be avoided.

Assay Procedure

BASIC INFORMATION, HANDLING AND TIPS:

The dots are precoloured blue on the strips, ensuring that all antigens have been dotted correctly onto the membrane. This blue coloration disappears during the first step of the incubation. During incubation with the wash buffer, a faint pink background coloration appears on the membrane and disappears upon drying at the end of the procedure. During the procedure, agitation of the incubation tray is necessary to ensure efficient circulation of fluids over the membrane. A Rocking platform is the shaker of choice. Be sure to adjust the movement of the shaker in such a way that no spilling of solutions or cross-contamination between the wells can occur. After each filling of the wells with solution, agitate manually the incubation tray until the strips are completely immersed in order to remove air bubbles which may be trapped under the strip. Alternatively, floating strips may be forced into the solution by pushing down (with tweezers or pipette tip) on the upper part of the strip (plastic label zone).

Avoid touching the membrane zone of the strip with fingers, tweezers or pipette tips. Always use the plastic label zone for handling or manipulation. The whole procedure has to be run at room temperature (18-25°C).

Description of the CONTROLS:

The Positive Control or RC (Reaction Control) consists of a protein fixing all the immunoglobulins present in the test sample. If the test has been carried out correctly, this control will show a colouring at the end of the test (with an intensity depending on the effective concentration of immunoglobulins in the sample). The absence of any colouring of this dot at the end of the test may indicate that the sample has not been pipetted on the strip.

The Negative Control or CO (Cut-Off Control) consists of a protein reacting with the enzymatic substrate and with certain constituent elements of the tested sample. If the test has been carried out correctly, this control is coloured at the end of the test, with a signal depending on the kinetics of the substrate and the characteristics of the sample. The intensity of this control serves as a threshold value for the final interpretation of the results.

Reagents preparation:

1. Allow all components to equilibrate at room temperature (18-25°C) before use.
2. Dilute the concentrated Wash Buffer 10× with distilled water. Prepare 15 ml diluted Wash buffer per strip tested. Example: 1.5 ml concentrated wash buffer + 13.5 ml distilled water for one strip.

Pipetting flow chart:

1. Place one strip per patient into the wells, blue dots facing up.
2. Add 2 ml Wash Buffer per well. Incubate (shake) for 10 min. Upon correct incubation, the blue coloration of the dots completely disappears. If not prolong the procedure until the colour of the dots fades completely.
3. Discard solution from the wells. Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.
4. Add 1.5 ml Sample Diluent per well.
5. Add 10 µl patient sample per well. Incubate (shake) for 30 min. Avoid touching the membrane with the pipette tip. Preferentially dispense the sample into the solution over the upper part of the strip (plastic label zone). **Note: Steps 4 and 5 can be combined by pre-diluting the sample in a glass or plastic tube (1.5 ml diluent + 10 µl patient sample). Mix (Add to the well)**
6. Discard solution from the wells. Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.
7. Wash 3 × 3 minutes with 1.5 ml Wash Buffer per well (shake). Following each wash step remove liquid from the wells by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the

tray with absorbent paper

8. Add 1.5 ml Conjugate per well. Incubate (shake) for 30 min.
9. Discard solution from the wells. Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper
10. Wash 3 × 3 min. with 1.5 ml Wash Buffer (shake). Following each wash step remove liquid from the wells by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the tray with absorbent paper.
11. Add 1.5 ml Substrate per well. Incubate (shake) for 10 min.
12. Discard solution from the wells. Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.
13. Wash 1 × 3 min. with 1.5 ml Wash solution per well to stop the reaction.
14. Collect the strips from the wells and allow them to dry for 30 minutes on absorbent paper. The interpretation has to be done in the 24 hours following the test processing.

Calculation

$$Result\ of\ antigen\ X\ (AU) = \frac{Grayscale\ intensity\ of\ antigen\ X - Grayscale\ intensity\ of\ CO}{Grayscale\ intensity\ of\ RC - Grayscale\ intensity\ of\ CO} * 100$$

AU: < 5 Negative

AU: 5-10 Equivocal (*)

AU: >10 Positive

Interpretation Of Results

1. Peel off the cover of the adhesive on the back side of each strip and attach strips dots face up onto the marked fields of the interpretation sheet provided with the kit. This will indicate the respective positions of the different controls and antigens on the membrane.
2. The first upper dot (Positive Control Dot) must be positive for all tests. Only a clearly coloured Positive Control Dot ensures your results are valid and operation was correct and/or kit components were not degraded. If the first upper dot is not coloured, the test has failed and cannot be interpreted further.
3. Compare the specific antigen dots to the Negative Control Dot (which always is the last bottom dot). The colour intensity of the antigen dots is directly proportional to the titer of the specific antibody in the sample. The colour intensity of the Negative Control Dot may vary depending on the sample characteristics. If the sample is free of interfering substances the Negative Control Dot may be even close to uncoloured. In contrast, a highly coloured Negative Control Dot indicates a high rate of unspecific binding in the sample.

POSITIVE RESULT:

A sample is positive for a specific antibody if the colour intensity of the corresponding Antigen dot is higher than the intensity of the Negative Control Dot.

NEGATIVE RESULT:

A sample is negative for a specific antibody if the colour intensity of corresponding Antigen dot is lower than or equal to the intensity of the Negative Control Dot.

Specificity

The main known interfering substances were tested on each biomarker of the present kit.

For each concentration of interfering substance tested, the difference between the result of the sample without the interfering substance and the result obtained in the presence of the interfering substance did not exceed 15%.

Interfering substance	Maximum Concentration	Intermediate Concentration	Minimum Concentration	Difference <15%
Bilirubin	100 mg/dL	50 mg/dL	25 mg/dL	Yes
Haemoglobin	200 mg/dL	100 mg/dL	50 mg/dL	Yes
Cholesterol	224.3 mg/dL	112 mg/dL	56 mg/dL	Yes
Rheumatoid factor IgM	~500IU/ml	~300IU/ml	~100IU/ml	Yes

Note: It is impossible to test all the possible interfering substances described in the literature. Other interferences, amongst others drug-induced interferences, are possible.

The high analytical specificity of the test is guaranteed by the quality of the antigen used. This kit detects IgG antibodies against β-Lactoglobulin (cow's milk) and Soya. No cross reactions with other autoantibodies have been found.

Precautions

1. The test kit should be processed by trained technical staff only.
2. The reagents in the kit are considered as not dangerous, as the concentrations of potentially dangerous chemicals are below the thresholds specified by European regulations (see MSDS). Nevertheless, the product contains preservatives which may have (in their given concentration), slightly polluting properties or causing skin sensitization. Therefore, contact with the skin, eyes or mucous membranes should be avoided. As with any chemical containing specific hazards, the product/components of the product should only be handled by qualified personnel and with the necessary precautions.
3. Samples should be handled as if they were capable of transmitting infectious diseases; they therefore require suitable protection (gloves, laboratory coat, goggles). In any case, GLP should be applied with all the general or individual safety rules in force.
4. Waste disposal: samples, incubated test strips and used reagent vials should be handled as infectious waste. The boxes and other containers do not need to be collected separately, unless stated otherwise in official regulations.