Version 18-04/25

User's Manual

Human ANCA Combi ELISA Kit

REF **DEIA-XY97**

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 test system provides detection of human antibodies of the immunoglobulin class IgG against six different antigens (proteinase 3, lactoferrin, myeloperoxidase, neutrophil elastase, cathepsin G and BPI (bactericidal/permeability increasing protein)).

Principles of Testing

The test kit contains microplate strips each with 8 reagent wells separately coated with these six antigens. In the first analysis step, the reagent wells are incubated with diluted samples. In the case the analyte is present in the sample, specific IgG antibodies (also IgA and IgM) will bind to the antigens. In a second analysis step, the bound antibodies are detected by incubating the samples with an enzyme-labeled antibody against human IgG (enzyme conjugate), which catalyzes a color reaction.

Reagents And Materials Provided

1. Antigen-coated reagent wells, 12 microplate strips each containing 8 wells in a frame, coated with 1. no antigen, 2. mixed antigen, 3. proteinase 3, 4. lactoferrin, 5. myeloperoxidase; 6. neutrophil elastase, 7. Cathepsin G, 8. BPI (≤150 µl/well), ready for use. 12 × 8

2. Calibrator, contains human serum/plasma with ANCA Profile IgG (1.0 ratio) in buffer solution, ready for use. Preservative: sodium azide <0.1% (w/w). 1 × 2.0 ml.

3. Enzyme conjugate, peroxidase-labeled antibody against human IgG (≤0.25%) in buffer solution, ready for use. Preservative: 2-methylisothiazol-3(2H)-one hydrochloride <0.1% (w/w). 1 × 12 ml

4. Sample buffer, buffer solution, ready for use. Preservative: sodium azide <0.1% (w/w). 1 × 100 ml

5. Wash buffer, buffer solution, 10× concentrated. Preservative: sodium azide <0.1% (w/w). 1 × 100 ml

6. Chromogen/substrate solution, TMB ≤0.04% (w/w), H_2O_2 ≤0.05% (v/v) in buffer solution, ready for use. 1 × 12 ml.

7. Stop solution, 0.5 M sulphuric acid, ready for use. 1 × 12 ml

8. Quality control certificate, 1 protocol.

Materials Required But Not Supplied

The list below shows instruments and materials that are required for the test performance, but not provided in the test kit. These articles can usually also be substituted by equivalent research articles.

1. Microplate reader with evaluation software: wavelength 450 nm, reference wavelength from 620 nm to 650 nm
2. Optional: Automatic microplate washer (Washing of the microplates can also be carried out manually.), Calibrated pipettes, Pipette tips
3. Optional: Water bath (recommended to warm the wash buffer), Distilled or deionized water, Vibration shaker

(vortex), Stopwatch, Disposable absorbent material (e.g. tissue paper)

Storage

The product has to be stored at +2 °C to +8 °C; do not freeze. Unopened, the product is stable until the indicated expiry date. Do not use the product after the expiry date.

In-use stability

After initial opening, the product is stable until the indicated expiry date when stored at +2 °C to +8 °C and protected from contamination, unless stated otherwise below.

Specimen Collection And Preparation

Sample preparation

The samples to be investigated are diluted 1:101 with sample buffer.

For example: pipette 1.0 ml sample buffer, add 10 µl sample and mix thoroughly (vortex). Sample pipettes are not suitable for mixing.

The calibrator is ready for use, do not dilute it.

Plate Preparation

Coating
A: no antigen
B: mixed antigen
C: PR3
D: Lactoferrin
E: MPO
F: Elastase
G: Cathepsin G
H: BPI

	1	2	3	4	5	6	7	8	9	10	11	12
A	BI	BI	BI	BI								
B	C	C	C	C								
C	P 1	P 2	P 3	P 4								
D	P 1	P 2	p 3	P 4								
E	P 1	P 2	P 3	P 4								
F	P 1	P 2	P 3	P 4								
G	P 1	P 2	P 3	P 4								
H	P 1	P 2	P 3	P 4								

The pipetting protocol for microplate strips 1 to 4 is an example for the ratio-based analysis of 4 samples (P 1 to P 4).

Sample buffer (BI) and the calibrator (C) have been incubated in the corresponding well of each microplate strip. The samples have been incubated in one well for each antigen.

The calibrator (C), sample buffer (BI) and the samples are all used in single determination. The reliability of the ELISA test can be improved by duplicate determination for each sample.

Reagent Preparation

All reagents must be brought to room temperature (+18 °C to +25 °C) before use.

The reagents can be removed from the test kit to accelerate acclimatization.

Sample buffer

Ready for use.

Coated wells

Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).

Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2 °C to +8 °C for 4 months.

Calibrator

Ready for use. The calibrator must be mixed thoroughly before use.

Wash buffer

The wash buffer is a 10× concentrate. If crystallization occurs in the concentrated buffer, warm it to +37 °C and mix well before diluting. The required volume must be diluted 1:10 with deionized or distilled water (1 part wash buffer plus 9 parts distilled water).

For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.

The working-strength wash buffer is stable for 4 weeks when stored at +2 °C to +8 °C and handled properly.

Enzyme Conjugate

Ready for use. The enzyme conjugate must be mixed thoroughly before use.

Chromogen/substrate solution

Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is colored blue.

Stop solution

Ready for use.

Assay Procedure

See quality control certificate for a schematic overview.

1. Sample incubation: (1st step)

Transfer 100 µl of the calibrator, the sample buffer (blank) or diluted samples into the individual microplate wells according to the pipetting protocol.

Incubate for 30 minutes at room temperature (+18 °C to +25 °C).

2. Washing:

Manual:

Empty the wells and subsequently wash 3 times using 300 µl of working-strength wash buffer for each wash.

Automatic:

Wash the reagent wells 3 times with 450 µl of working-strength wash buffer.

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on

disposable absorbent material with the openings facing downwards to remove all residual wash buffer.

Caution:

Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

3. Conjugate incubation: (2nd step)

Pipette 100 µl of enzyme conjugate (peroxidase-labeled antibody against human IgG) into each of the microplate wells. Incubate for 30 minutes at room temperature (+18 °C to +25 °C).

4. Washing:

Empty the wells. Wash as described above.

5. Substrate incubation: (3rd step)

Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for 15 minutes at room temperature (+18 °C to +25 °C) (protect from direct sunlight).

6. Stopping:

Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

7. Measurement:

Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Calculation

Ratio-based

If the photometer has no automatic blank adjustment, Creative Diagnostics recommends to first calculate the mean blank value and to subtract from all other measured values. Then the mean extinction value for all measurements of the calibrator is calculated and multiplied by the factor 0.2. This provides the upper limit of the normal range (cut-off). Values above the indicated cut-off are to be considered as detected, those below as not detected.

The test can be evaluated by calculating a ratio of the extinction value of the sample over the extinction value of the cut-off. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the sample}}{\text{Cut-off extinction}} = \frac{\text{Extinction of the sample}}{\text{Extinction of calibrator x 0.2}} = \text{Ratio}$$

Exception PR3: For PR3 the calculated ratio is multiplied by the factor 1.4.

$$\frac{\text{Extinction of the sample}}{\text{Cut - off extinction}} = \frac{\text{Extinction of the sample}}{\text{Extinction of calibrator x 0.2}} = \text{Ratio x 1.4}$$

Creative Diagnostics recommends evaluating results as follows:

Not detected: Ratio <1.0

Detected: Ratio ≥1.0

For duplicate determinations the mean of the two values should be taken.

Precautions

1. The product must only be used by trained laboratory personnel in a research laboratory.
2. Do not use the product if the packaging of the reagents is damaged.
3. Before using the product, read the instructions for use carefully.
4. Sample buffer, wash buffer, enzyme conjugate, chromogen/substrate solution and stop solution are interchangeable between lots if they have identical article numbers (see labeling). All other reagents are lot-specific. Mixing or replacing by reagents from other lots or manufacturers is not recommended.
5. Observe laboratory and safety guidelines. The product contains preservatives.
6. The calibrator of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all test kit components should be treated as potentially infectious and handled with care.
7. Waste disposal: Samples, calibrator and incubated microplate strips should be handled as infectious waste. All products must be disposed of in accordance with legal regulations.

Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. It is the responsibility of laboratories to define and validate their own experimental design and analysis parameters.
3. Partial or complete adaptation of the test system for use with automated sample processors or other liquid handling devices may lead to differences between the results obtained with the automated and manual procedure. It is the responsibility of the user to validate the automated instruments used for the analysis to ensure that they yield test results within the permissible range.
4. Insufficient washing (e.g. less than 3 wash cycles, too small wash buffer volumes or too short residence times) can lead to false extinction readings.
5. Residual liquid (>10 µl) in the reagent wells after washing can interfere with the substrate conversion and lead to false low extinction readings.
6. Correct sample collection and storage are crucial for the reliability of the test results.
7. The binding activity of the antibodies and the activity of the enzyme used are temperature dependent. It is therefore recommended to use a thermostatically controlled ELISA incubator in all incubation steps.