Calprotectin (Serum) ELISA Kit

Cat. No.:DEIA2166
Pkg.Size:96T

Intended use

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended measurement of Calprotectin (MRP8/14) in serum, plasma and urine.

General Description

Measurement of faecal calprotectin is a biochemical test for inflammatory bowel disease. It replaces the need for invasive colonoscopy or radio-labelled white cell scanning in many clinical scenarios.

Principle Of The Test

The assay utilizes the two-site "sandwich" technique with two selected monoclonal antibodies that bind to human Calprotectin. Standards, controls and diluted specimen samples which are assayed for human Calprotectin are added to wells of microplate coated with a high affine monoclonal anti-human Calprotectin antibody. During the first incubation step, Calprotectin in the samples is bound by the immobilized antibody. In a next incubation step, a biotinylated monoclonal anti-human Calprotectin antibody is added to each microtiter well. Then a peroxidase labeled exravidin conjugate is added to each well and the following complex is formed: capture antibody - human Calprotectin – biotinylated detection antibody - Peroxidase conjugate. Tetramethylbenzidine (TMB) is used as a substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the Calprotectin concentration of sample. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from standard. Calprotectin present in the specimen samples, is determined directly from this curve.

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. Detection antibody, (monoclonal anti-Calprotectin (MRP 8/14) antibody, biotinylated), concentrate: 50 μL
4. Calprotectin standards, lyophilized (0; 3.9; 15.6; 62.5; 250 ng/mL): 2 x 5 vials
5. Control, lyophilized (see specification for range): 2 x 1 vial
6. Control, lyophilized (see specification for range): 2 x 1 vial
7. Conjugate, (extravidin peroxidase labeled), concentrate: 50 μL
8. TMB substrate (Tetramethylbenzidine), ready to use: 15 mL
9. ELISA stop solution, ready to use: 15 mL

Materials Required But Not Supplied

1. Ultra pure water
2. Laboratory balance
3. Precision pipettors calibrated and tips to deliver 10-1000 μL
4. Covering foil for the microtiter plate
5. Horizontal microtiter plate shaker with 37 ℃ incubator
6. A multi-channel dispenser or repeating dispenser

7. Centrifuge capable of 3000 x g

8. Vortex-Mixer

9. Standard laboratory glass or plastic vials, cups, etc.

10. Microtiter plate reader at 450 or 405 nm

Storage

Store all contents at 2 to 8 ℃.

Specimen Collection And Handling

1. Preanalytic handling: Significant differences in the calprotectin levels can be observed due to different sample preparation procedures, e. g. up to 10-fold higher serum levels compared to the plasma calprotectin concentrations. The reasons are as follows:

Granulocytes are activated during serum clotting and release granulocyte-activating markers. The time between serum collecting and analysis as well as repeated freeze-thaw cycles don't cause a calprotectin concentration shift. On the contrary, in the case of plasma samples, varying the time between sampling and analysis or the number of freeze- thaw cycles will cause variation in the observed calprotectin levels. Therefore, the preanalytical conditions of plasma samples should be held constant. This is a general requirement independent of the used test-system. The use of serum samples for calprotectin determinations is recommended.

2. Serum samples should be diluted 1:50 with wash buffer before assaying.

3. EDTA Plasma samples should be diluted 1:10 with wash buffer before assaying.

4. Urine samples should be diluted 1:10 with wash buffer before assaying.

Reagent Preparation

1. To run assay more than once, ensure that reagents are stored at the conditions stated on the label. Prepare only the appropriate amount necessary for each assay. 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 with ultra pure water 1:10 before use (100 mL concentrate add with 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 room temperature or at 37 ℃ using a water bath before dilution of the buffer solutions.

4. The buffer concentrate is stable at 2 ℃ - 8 ℃ until the expiry date stated on the label.

5. Diluted buffer solution can be stored in a closed flask at 2 ℃ - 8 ℃ for one month.

6. The lyophilized standards and controls are stable at 2 ℃ - 8 ℃ until the expiry date stated on the label. The standards) and controls must be reconstituted with 500 μL ultra pure water Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution.

7. Reconstituted standards and control can be stored at 2 ℃ - 8 ℃ for four weeks.

8. The detection antibody (AB) must be diluted 1:1000 in wash buffer (10 μL AB add with 10 mL wash buffer). The antibody is stable at 2 ℃ - 8 ℃ until expiry date given on the label. Diluted antibody solution is not stable and could not be stored.

9. The conjugate must be diluted 1:1000 in wash buffer (10 μL conjugate add with 10 mL wash buffer). The antibody is stable at 2 ℃ - 8 ℃ until expiry date given on the label. Diluted conjugate is not stable and can not be stored.

10. All other test reagents are ready to use. The test reagents are stable until the expiry date (see label of test package) when stored at 2 ℃ - 8 ℃.

Assay Steps

1. Bring all reagents and samples to room temperature (18 ℃ - 26 ℃) and mix well

2. Mark the positions of STD /SAMPLE/CTRL (Standards/Sample/Control) in duplicate on a protocol sheet

3. Take as many microtiter strips as needed from kit. Store unused strips covered at 2 ℃ - 8 ℃. Strips are stable until expiry date stated on the label

4. Add 100 µL of STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective well

5. Cover plate tightly and incubate for 1 hour at 37 ℃ on a horizontal mixer

6. Aspirate the contents of each well. Wash 5 times by dispending 250 µL of Wash buffer into each well. After the final washing step, the inverted microtiter plate should be firmLy tapped on absorbent paper

7. Add 100 µL AB (detection antibody) into each well

8. Cover plate tightly and incubate for 1 hour at 37 ℃ on a horizontal mixer

9. Aspirate the contents of each well. Wash 5 times by dispending 250 µL of Wash buffer into each well. After the final washing step, the inverted microtiter plate should be firmLy tapped on absorbent paper

10. Add 100 µL CONJ (conjugate) into each well

11. Cover plate tightly and incubate for 1 hour at 37 ℃ on a horizontal mixer

12. Aspirate the contents of each well. Wash 5 times by dispending 250 µL of Wash buffer into each well. After the final washing step the inverted microtiter plate should be firmLy tapped on absorbent paper

13. Add 100 µL of SUB (substrate) into each well

14. Incubate for 10 - 20 minutes at room temperature (18 ℃ - 26 ℃) in the dark

15. Add 100 µL of STOP (stop solution) into each well, mix thoroughly

16. Determine absorption immediately with an ELISA reader at 450 nm. If the highest extinction of the standards (STD) is above the range of the photometer, absorption must be measured immediately at 405 nm and the obtained results used for evaluation. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e. g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used.

Quality Control

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. We recommend each laboratory to establish its own norm concentration range.

Calculation

The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-Parameter- algorithm".

1. 4-parameter-algorithm: It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001).

2. Point-to-point-calculation: We recommend a linear ordinate for optical density and a linear abscissa for concentration.

3. Spline-algorithm: We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001). The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

4. Serum: For calculation of calprotectin concentration in serum, the result must be multiplied by 50.

5. EDTA Plasma: For calculation of calprotectin concentration in plasma, the result must be multiplied by 10.

6. Urine: For calculation of calprotectin concentration in urine, the result must be multiplied by 10.

Detection Range

3.9 - 250 ng/mL

Sensitivity

3.2 ng/mL

Precautions

Quality control guidelines should be observed. 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. Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes. Stop solution contains sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.

Limitations

Samples with Calprotectin levels greater than the highest standard value, should be further diluted with wash buffer, and re-assayed.

REFERENCES

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