Secretory IgA ELISA Kit

Cat. No.:DEIA2003
Pkg.Size:96T

Intended use

The Assay is intended for determination of secretory IgA (sIgA) in sputum and stool.

Principle Of The Test

This Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) is intended for the determination of secretory IgA in stool and saliva. In a first incubation step, the sIgA in the samples is bound to polyclonal antibodies (rabbit anti human IgA), which are immobilized to the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a Peroxidase-labeled conjugate (mouse anti-sIgA) is added which recognizes specifically the bound secretory IgA. After another washing step, to remove all unbound substances, the solid phase is incubated with the substrate, Tetramethylbenzidine (TMB). An acidic stop solution is then added to stop the reaction. The color converts from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of secretory IgA. A dose response curve of the absorbance unit (optical density, OD) vs. concentration is generated, using the results obtained from the calibrators. Secretory IgA in the 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: 1 x 100 ml
3. Conjugate (mouse anti-sIgA, Peroxidase-labeled): 1 x 200 µl
4. Calibrators, lyophilized (0; 22.2; 66.6; 200; 600 ng/ml): 2 x 5 vials
5. Control 1, lyophilized: 2 x 1 vial
6. Control 2, lyophilized: 2 x 1 vial
7. TMB Substrate (Tetramethylbenzidine), ready-to-use: 1 x 15 ml
8. ELISA Stop Solution, ready-to-use: 1 x 15 ml

Materials Required But Not Supplied

1. Ultra pure water* 
2. Laboratory balance
3. Precision pipettors and disposable tips to deliver 10-1000 µl
4. Foil to cover the microtiter plate
5. Horizontal microtiter plate shaker
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 nm (reference wave length 620 or 690 nm)

*CD recommends the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25°C (≤18.2 MΩ cm).

Specimen Collection And Handling
Saliva
To avoid variation in sIgA content, take saliva samples always at the same time of the day.
No food or liquid should be consumed 30 min before sample collection. Centrifuge the samples at 3000 rpm for 10 min. Sample supernatant can be stored at -20°C.
For analysis, the supernatant is diluted 1:2000 in ELISA wash buffer, e.g. 10 µl supernatant + 990 µl wash buffer; dilute the obtained solution again: 50 µl diluted supernatant + 950 µl wash buffer.
Use 100 µl of the final dilution per well.

Faeces
1. Extraction of stool samples
1a. Stool Sample Application System (SAS)
Stool sample tube – Instruction for use
Please note that the dilution factor of the final stool suspension depends on the used amount of stool sample and the volume of the buffer.
SAS with 0.75 ml Buffer:
Applied amount of stool: 15 mg
Buffer Volume: 0.75 ml
Dilution Factor: 1:50
Wash buffer is used as extraction buffer.
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 remarkably inhomogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.
b. Fill the empty sample tube with 0.75 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 exhibits 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 and leave 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 app. 10 minutes until sediment has settled down. 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, the sediment will not be dispersed again.
1b. Sample preparation kit from Roche Diagnostics, Mannheim, Germany
Alternatively, other stool sample preparation kits (e.g. Sample preparation kit from Roche Diagnostics, Mannheim, Germany) can be used. In the Roche sample preparation kit, 100 mg of stool sample are suspended in 5 ml of extraction buffer using a vibrator mixer (e.g. Vortex mixer). Centrifugation of the suspension is recommended.
Dilution Factor (1a. or 1b.): 1:50
2. Dilution of samples Stool samples
After centrifugation, the supernatant is diluted 1:250 in wash buffer.
For example:
40 µl supernatant + 960 µl wash buffer, mix well (dilution I) (1:25)
100 µl of this dilution I + 900 µl wash buffer, mix well (dilution II) (1:10)
For analysis, pipette 100 µl of dilution step II solution per well.
Reagent Preparation

1. To run assay more than once, ensure that reagents are stored at 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. The ELISA WASHBUF (wash buffer concentrate) should be diluted with ultra pure water 1:10 before use (100 ml WASHBUF + 900 ml ultra pure water). Crystals could occur due to high salt concentration. The crystals must be resuspended before dilution of the buffer solutions using a water bath (37°C). The buffer concentrate is stable at 2-8°C until the expiry date stated on the label. Diluted solutions can be stored at 2-8°C for 1 month.

3. The STD (standards) and CTRL (control) 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 ensure complete reconstitution. Reconstituted standards and control are stable at -20 °C until the expiry date stated on the label and can be subjected to a maximum of two freeze-thaw cycles.

4. The CONJ (conjugate; POD-labeled antibody) must be diluted 1:101 in wash buffer (100 μl CONJ + 10 ml wash buffer). The undiluted conjugate is stable at 2-8 °C until the expiry date stated on the label. Diluted conjugate is not stable and cannot be stored.

5. All other test reagents are ready for use. The test reagents are stable up to the date of expiry (see label of test package) when stored at 2 – 8 °C.

Assay Steps

Procedural notes

1. Do not mix different lot numbers of any kit component.
2. Quality control guidelines should be followed.
3. Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. CD can therefore not be held responsible for any damage resulting from wrong use.

4. The assay should always be performed according to the enclosed manual.

Test procedure

Wash the precoated PLATE (microtiter plate) 5 x with 250 μl ELISA wash buffer. Carry out the tests in duplicate.

1. Add 100 μl STD (standards), CTRL (controls) and samples (faeces and saliva diluted, see above).
2. Incubate for 1 hour, shaking on a horizontal mixer, at room temperature.
3. Aspirate and wash the wells 5 x with 250 μl ELISA wash buffer.
4. Add 100 μl CONJ (conjugate; POD antibody).
5. Incubate for 1 hour, shaking on a horizontal mixer, at room temperature.
6. Decant the content of the plate and wash the wells 5 x with 250 μl wash buffer.
7. Add 100 μl SUB (TMB substrate).
8. Incubate for 10-20 minutes at room temperature.
9. Add 50 μl STOP (ELISA stop solution) and mix shortly.
10. 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. The results for the samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.
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.

Saliva

For the calculation of the saliva values the results from the microplate reader has to be multiplied with 2.000.

Faeces

A final dilution factor of 1:12500 results for a constant dilution factor 50 in dilution step 1:

Dilution step 1: 50
Dilution step 2: 250

Final dilution factor: 50 x 250 = 12500

Precautions

1. 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.

2. Stop solution is composed of 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.

3. Reagents should not be used beyond the expiration date shown on the kit label

Limitations

Samples with sIgA levels greater than the highest calibrator, should be diluted and re-assayed.

REFERENCES


