Product information

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Instructions for use

Secretory IgA ELISA

A solid-phase enzyme immunoassay for the quantitative determination of secretory IgA in human biological fluids

CE

IVD

REF **DEXK276**

Σ **96 Tests**

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1. INTENDED USE

A solid-phase enzyme immunoassay for the quantitative determination of secretory IgA in biological fluids.
This kit is designed for measurement of secretory IgA in biological fluids. For possibility of use with other sample types, please, refer to Application Notes (on request). The kit contains reagents sufficient for 96 determinations and allows to analyze 41 unknown samples in duplicates.

2. SUMMARY AND EXPLANATION

Secretory IgA (sIgA) is the main immunoglobulin present on mucosal surfaces. Ca. 90% of sIgA is produced locally and does not penetrate into blood circulation. sIgA is considerably different from serum IgA, as this complex protein consists of 3 completely different molecules. Two or four molecules of immunoglobulin A with molecular weight 160 kDa are joined by J-chain (16 kDa) and attached to the secretory component (80kDa); the formation of this complex occurs during transepithelial transport of polymeric IgA.
sIgA plays a pivotal role in local immunity by blocking bacterial and viral adhesion and invasion through epithelial tissues. Determination of sIgA concentration allows to evaluate the local immunity status in stomatology, ophthalmology, respiratory diseases, gastroenterology, gynaecology. The sIgA in saliva can be also used as noninvasive mass screening for selective IgA deficiency.
Elevation of sIgA in serum is occasionally observed in so autoimmune diseases and several tumours.

3. PRINCIPLE OF THE TEST

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal to human secretory IgA-antibodies. Antigen from the specimen is captured by the antibodies coated onto the microwell surface. Unbound material is removed by washing procedure. Second antibodies - murine monoclonal to human IgA alpha chain, labelled with peroxidase enzyme, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

4. WARNINGS AND PRECAUTIONS

4.1. This kit is intended for in vitro diagnostic use only.
4.2. INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.
4.3. Avoid contact with stop solution containing acidic solution. It may cause skin irritation and burns.
4.4. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.
4.5. Do not use the kit beyond the expiration date.
4.6. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.
4.7. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
4.8. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.
4.9. Do not mix reagents from different lots.
4.10. Replace caps on reagents immediately. Do not swap caps.
4.11. Do not pipette reagents by mouth.
4.12. Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.
4.13. Safety Data Sheet for this product is available upon request directly from Demeditec Diagnostics GmbH.
4.14. The Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

5. KIT COMPONENTS

5.1. Contents of the Kit

	Symbol	*Description*		*Qty*	*Units*	*Colour code*	*Stability of opened/diluted components*
1	SORB MTP	secretory IgA EIA strips, 8x12 wells	polystyrene microwells coated with murine monoclonal to human secretory IgA	1	pcs		until exp.date
2	CAL 1 - 6	Calibrator set, 1 ml each. The set contains 6 calibrators: 0; 2; 20; 40; 100, 400 µg/ml	human secretory IgA diluted in tris buffered BSA solution, preservative - 0,01% Bronidox L, 0,01% 2-Methyl-4-isothiazolin-3-one-hydrochloride; also contains bright blue dye	6	pcs	bright blue (C1 - colourless)	2 months
3	CONTROL	Control serum (1 ml)	dilution of preselected human serum, with high content of secretory IgA with BSA solution; preservative - 0,01% Bronidox L, 0,01% 2-Methyl-4-isothiazolin-3-one-hydrochloride, colourless	1	pcs	colourless	2 months
4	CONJ HRP	Conjugate, 11 ml	aqueous solution of murine monoclonal to human IgA alfa chain coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0,1% phenol as preservative and bright red dye	1	pcs	bright red	until exp.date
5	DIL	red EIA buffer 22 ml	phosphate buffered saline with casein from bovine milk and detergent (Tween-20), contains 0,1% phenol as preservative; contains red dye	1	pcs	red	until exp.date
6	DIL SPE	EIA buffer 100 ml	phosphate buffered saline with casein from bovine milk and detergent (Tween-20), contains 0,1% phenol as preservative and blue dye	1	pcs	blue	until exp.date
7	SUBS TMB	Substrate solution, 11 ml	ready-to-use single-component tetramethylbenzidine (TMB) solution.	1	pcs	colourless	until exp.date
8	BUF WASH 21X	Washing solution concentrate 21x, 22 ml	aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative	1	pcs	colourless	Concentrate - until exp.date Diluted washing solution - 1 month at 2-8 °C or 5 days at RT
9	STOP	Stop solution, 11 ml	5,0% vol/vol solution of sulphuric acid	1	pcs	colourless	until exp.date
10	N003	Plate sealing tape		2	pcs		N/A
11	K276I	Instruction secretory IgA EIA		1	pcs		N/A
12	K276Q	QC data sheet secretory IgA EIA		1	pcs		N/A

5.2 Equipment and material required but not provided
- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100-250 µl, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 25-250 µl;
- Dry thermostat for 37°C +/- 0.1°C
- Calibrated microplate photometer with 450 nm wavelength and OD measuring range 0-3.0.

5.3. Storage and stability of the Kit
Store the whole kit at 2 to 8 °C upon receipt until the expiration date. After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

6. SPECIMEN COLLECTION AND STORAGE.

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.
Specimens may be stored for up to 48 hours at 2-8 °C before testing. Calibrators and control sample(s) - only one freezing/thawing cycle is allowed

7. TEST PROCEDURE

7.1. Reagent Preparation
- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18 to +25°C) before use.
- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 21X by 21 dilutions in distilled water.

7.2. Procedural Note: It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.
7.3. Assay flowchart. See the example of calibration graphic in Quality Control data sheet.
7.4. Assay procedure

1	Put the desired number of microstrips into the frame; allocate 14 wells for the calibrators CAL 1 - 6 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS. NOTE: the calibrator/control and unknown sample wells are filled differently.
2	Dilute samples using buffer DIL SPE (EIA buffer) 101 fold. See table M for dilution modes and factors for different types of analyzed material. Do not dilute control sample and calibrators.
3	If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using DIL SPE (EIA buffer). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.
4	Pipet 190 µl of red EIA buffer into the wells allocated for saliva. For other tested materials, see table M for the volume of red EIA buffer.
5	Pipet 100 µl of calibrators CAL 1 - 6 and control samples CONTROL into allocated wells. For testing of saliva pipet 10 µl of the unknown sample into the allocated wells. See table M for the volumes of other materials. Carefully mix the contents of the wells by short horizontal rotating of the plate for 5-7 seconds and cover the wells by plate adhesive tape (included into the kit).
6	Incubate 90 minutes at 37 °C .
7	Prepare washing solution by 21x dilution of washing solution concentrate BUF WASH 21X by distilled water. Minimal quantity of washing solution should be 250 µl per well. Wash strips 3 times
8	Dispense 100 µl of CONJ HRP into the wells. Cover the wells by plate adhesive tape.
9	Incubate 30 minutes at 37 °C .
10	Wash the strips 5 times.
11	Dispense 100 µl of SUBS TMB into the wells
12	Incubate 10-20 minutes at 18-25 °C
13	Dispense 100 µl of STOP into the wells.
14	Measure OD (optical density) at 450 nm.
15	Set photometer blank on first calibrator
16	Apply point-by-point method for data reduction. Use Calculation factor listed in table M to calculate analyte concentration in different material types.

7.5. Handing notes
Calibrators and control sample(s) - only one freezing/thawing cycle is allowed

7.6. Sample processing (Table M)

Material type	Notes on material collection, storage and handling	Sample dilution example	red EIA buffer into the well, µl	Sample into the well, µl	Calculation factor
blood serum or plasma	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.	5 µl of sample + 500 µl of diluent	0	100	0.05
saliva	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.	5 µl of sample + 500 µl of diluent	190	10	1.0
urine	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.	10 µl of sample + 500 µl of diluent	0	100	0.025
bronchoalveolar fluid	Turbid samples should give incorrect measurement results and should be treated by centrifugation before testing.	5 µl of sample + 500 µl of diluent	0	100	0.05
nasal wash	Turbid samples should give incorrect measurement results and should be treated by centrifugation before testing.	5 µl of sample + 500 µl of diluent	80	20	0.25
vaginal secret	Turbid samples should give incorrect measurement results and should be treated by centrifugation before testing.	5 µl of sample + 500 µl of diluent	90	10	0.5
breast milk	Turbid samples should give incorrect measurement results and should be treated by centrifugation before testing.	5 µl of sample + 2500 µl of diluent	195	5	10

8. QUALITY CONTROL

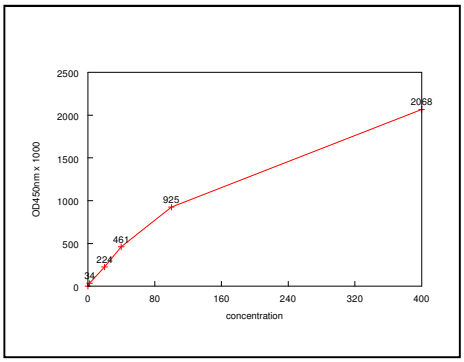
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.
The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

9. CALCULATION OF RESULTS

9.1. Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.
9.2. Plot a calibration curve on graph paper: OD versus secretory IgA concentration.
9.3. Determine the corresponding concentration of secretory IgA in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

9.4. Below is presented a typical example of a standard curve with the Demeditec Assay. Not for calculations!

Calibrators	Value	Absorbance Units (450 nm)
CAL 1	0 µg/ml	0.10
CAL 2	2 µg/ml	0.14
CAL 3	20 µg/ml	0.33
CAL 4	40 µg/ml	0.57
CAL 5	100 µg/ml	1.03
CAL 6	400 µg/ml	2.17



10. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for secretory IgA. Based on data obtained by Demeditec, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

Sex, age	Units	
	µg/ml	
	Lower limit	Upper limit
serum	1.6	5.0
saliva	57	260
urine	0.5	2.7
breast milk	800	-

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical specificity / Cross reactivity

Analyte	Cross-reactivity, % wt/wt
IgG	<0.1
IgM	<0.1
IgE	<0.1

11.2. Analytical sensitivity: Sensitivity of the assay was assessed as being 0.6 µg/ml.
11.3. Linearity was checked by assaying dilution series of 5 samples with different secretory IgA concentrations. Linearity percentages obtained ranged within 90 to 110%.
11.4. Recovery was estimated by assaying 5 mixed samples with known secretory IgA concentrations. The recovery percentages ranged from 90 to 110%.

12. LITERATURE

1. Heiddis B. Valdimarsdottir and Arthur A. Stone - Psychosocial Factors and Secretory Immunoglobulin A. Critical Reviews in Oral Biology & Medicine, Jan 1997; 8: 461 - 474.
2. Amir H Abdul Latiff and Michael A Kerr - The clinical significance of immunoglobulin A deficiency. Ann Clin Biochem, Mar 2007; 44: 131 - 139.