Anti-SS-B-La (IgG) ELISA

For the quantitative and qualitative detection of antibodies against La-antigen / SS-B in serum

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 35-SSBHU-E01
Size: 96 wells
Version: 004 : 2017-08-16 - ALPCO June 5, 2018

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1. Intended Use

SS-B (IgG) ELISA is a solid phase enzyme immunoassay employing human recombinant La-antigen/ SS-B for the quantitative and qualitative detection of antibodies against La-antigen / SS-B in human serum.

2. Principle of the Assay

SS-B is a 48 kDa protein associated with RNA polymerase III which seems to assemble with all precursor RNAs transcribed by polymerase III. Numerous functions have been assigned for the SS-B protein including a role in transcription/termination of RNA synthesized by polymerase III, 3`RNA processing and nuclear import and retention. Furthermore, it has been proposed to be an RNA chaperone which is involved in the stabilization of RNA secondary structure.

2.1. Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Antibodies, if present in the sample, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards, anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the sample.

3. Kit Contents

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20mL	White	Yellow	5x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 X 20mL	White	Green	50x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5mL	Green	Colorless	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5mL	Red	Yellow	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	1 x 1.5mL	Blue	Yellow	Calibrator material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5mL	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/mL. Calibrator material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)

Conjugate, IgG	1 x 15mL	Blue	Blue	Containing: Immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15mL	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15mL	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glassware (cylinder 100-1000mL), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µL) or adjustable multipipette (100-1000µL). Microplate washing device (300 µL repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

5. Precautions of Use

5.1. Health hazard data

THIS PRODUCT IS FOR RESEARCH USE ONLY.

Recommendations and precautions
This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin, it is recommended to avoid contact with eyes and skin and wear disposable gloves.

WARNING! Calibrators, Controls and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or absorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All biologic source material used for some reagents of this kit has been tested by approved methods and found negative for HBsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus, handle these reagents as if capable of transmitting infectious diseases and according to national requirements.

The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.

5.2. General directions for use

- In case that the product information, including the labeling, is defective or incorrect please contact the manufacturer or the supplier of the test kit.
- Do not mix or substitute Controls, Calibrators, Conjugates or microplates from different lot numbers. This may lead to variations in the results.
- Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.
- **Incubation: It is recommended to set automated systems to 30°C/86°F.**
- Never expose components to higher temperature than 37°C/ 98.6°F.
- Always pipette substrate solution with brand new tips only. Protect this reagent from light.
- Never pipette conjugate with tips used with other reagents prior.

6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolyzed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 xg). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum samples should be used during the first 8 hours, respectively stored tightly closed at 2-8°C/35-46°F up to 48hours, or frozen at -20°C/-4°F for longer periods.

7. Assay Procedure

7.1. Preparations prior to starting

Dilute concentrated reagents:

- Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 mL plus 80 mL).
- Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 mL plus 980 mL).
- To avoid mistakes, it is suggested to mark the cap of the different calibrators.

Samples:
Dilute serum samples 1:101 with sample buffer (1x)
e.g. 1000 µL sample buffer (1x) + 10 µL serum
Mix well!

Washing:
Prepare 20 mL of diluted wash buffer (1x) per 8 wells or 200 mL for 96 wells
e.g. 4 mL concentrate plus 196 mL distilled water

Automated washing:
Consider excess volumes required for setting up the instrument and dead volume of robotic pipettes.

Manual washing:
Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µL of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplate:
Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

7.2. Pipetting Scheme

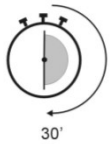
	1	*2*	*3*	*4…*
A	***Cal A***	***Cal E***	***S1***	
B	***Cal A***	***Cal E***	***S1***	
C	***Cal B***	***Cal F***	***S2***	
D	***Cal B***	***Cal F***	***S2***	
E	***Cal C***	***PC***	***S3***	
F	***Cal C***	***PC***	***S3***	
G	***Cal D***	***NC***	***…***	
H	***Cal D***	***NC***	***…***	

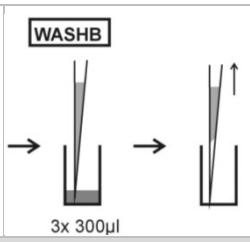
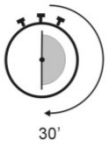
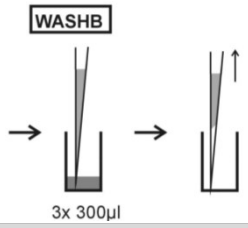
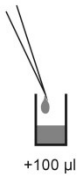
	1	*2*	*3*	*4…*
A	***NC***	***S2***		
B	***NC***	***S2***		
C	***CC***	***S3***		
D	***CC***	***S3***		
E	***PC***			
F	***PC***			
G	***S1***			
H	***S1***			

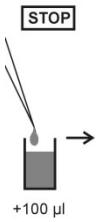
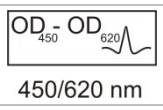
It is suggested to pipette calibrators, controls and samples in this manner.

For the Quantitative Interpretation		**For the Qualitative Interpretation**	
CalA: Calibrator A	CalD: Calibrator D	PC: Positive Control	S1: Sample 1
CalB: Calibrator B	CalE: Calibrator E	NC: Negative Control	S2: Sample 2
CalC: Calibrator C	CalF: Calibrator F	CC: Cut-off Calibrator	S3: Sample 3

7.3. Test Steps

Step	*Description*	
1.	Ensure preparations from step 7.1 above have been carried out prior to pipetting.	
2.	Use the following steps in accordance with quantitative/qualitative interpretation results desired:	
		CONTROLS & SAMPLES
3.		Pipette into the designated wells as described in step 7.2 above, 100 µL of either: a. Calibrators (CAL.A to CAL.F) for *QUANTITATIVE* or b. Cut-off Calibrator (CC) for *QUALITATIVE* interp. c. and 100 µL of each of the following: • Negative control (NC) and Positive control (PC), and • Diluted Sample serum (S1, S2...)
4.		Incubate for 30 minutes at 20-32°C/68-89.6°F.

5.		Wash 3x with 300 µL wash buffer (diluted 1:50).
	CONJUGATE	
6.		Pipette 100 µL conjugate into each well.
7.		Incubate for 30 minutes at 20-32°C/68-89.6°F.
8.		Wash 3x with 300 µL wash buffer (diluted 1:50).
	SUBSTRATE	
9.		Pipette 100 µL TMB substrate into each well.
10.		Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
	STOP	

11.		Pipette 100 µL stop solution into each well, using the same order as pipetting the substrate.
12.		Incubate 5 minutes minimum.
13.		Agitate plate carefully for 5 sec.
14.		Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

8. Quantitative and Qualitative Interpretation

For **quantitative interpretation** establish the standard curve by plotting the **optical density** (**OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in U/mL (x-axis). For best results, it is recommended to use log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/mL.

Example of a standard curve

It is recommended to pipette calibrators in parallel for each run.

Calibrators IgG	OD 450/620 nm	CV % (Variation)
0 U/mL	0.020	0.1
3 U/mL	0.124	2.8
10 U/mL	0.265	1.9
30 U/mL	0.565	2.0
100 U/mL	1.151	1.6
300 U/mL	2.134	0.9

Example of calculation

Sample	Replicate (OD)	Mean (OD)	Result (U/mL)
S 01	0.985/0.980	0.983	72.1
S 02	1.866/1.861	1.864	227.5

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.

For lot specific data, see enclosed quality control leaflet. Laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

In case that the values of the controls do not meet the criteria, the assay is invalid and has to be repeated. The following technical issues should be verified: expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods. If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact ALPCO.

For **qualitative interpretation** read the optical density of the cut-off calibrator and the samples. Compare sample OD with the OD of the cut-off calibrator. For qualitative interpretation, it is recommended to consider sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

Negative:		**OD sample**	**<**	**0.8 x OD cut-off**		
Equivocal:	**0.8 x**	**OD cut-off**	**≤**	**OD sample**	**≤**	**1.2 x OD cut-off**
Positive:		**OD sample**	**>**	**1.2 x OD cut-off**		

9. Technical Data

Sample material:	serum
Sample volume:	10 µL of sample diluted 1:101 with 1x sample buffer
Total incubation time:	90 minutes at 20-32°C/68-89.6°F
Calibration range:	0-300 U/mL
Analytical sensitivity:	1.0 U/mL
Storage:	at 2-8°C/35-46°F use original vials only.
Number of determinations:	96 tests

10. Performance Data

10.1. Precision

Precision of assay results obtained with the **SS-B (IgG) ELISA** were assessed by the determination of the intra- and inter assay precision as well as the lot-to-lot variance by the analysis of multiple sample of different antibody activities.

Sample ID	Intra-Assay Precision		Inter-Assay Precision		Lot-to-Lot Precision	
	Mean (U/mL)	CV (%)	Mean (U/mL)	CV (%)	Mean (U/mL)	CV (%)
Sample 1	8.03	8.9	8.12	18.6	8.12	7.1
Sample 2	12.94	11.2	13.07	12.6	13.07	12.1
Sample 3	42.17	11.7	42.17	15.3	41.53	11.0
Sample 4	81.71	9.7	81.71	11.2	79.56	10.01
Sample 5	149.21	8.5	147.71	14.8	146.10	12.3

10.2. Analytical sensitivity

The analytical sensitivity was assessed by multiple analysis of sample buffer and low positive samples and calculating the limit of detection. The analytical sensitivity of the SS-B (IgG) ELISA is 1.74 U/mL.

10.3. Linearity

Three sera covering the whole assay range were diluted serially with a negative serum sample. Measured and expected values of the distinct dilutions were used to calculate a linear regression. According to results of linearity testing a measurable range of 3-300 U/mL was determined for the SS-B (IgG) ELISA.

10.4. Calibration

Due the lack of international reference calibration this assay is calibrated in arbitary units (U/mL).

11. Disposal

Please observe the relevant statutory requirements.

12. Literature

1. Kalden JR (1988). Sjögren-Syndrom. In Kalden JR (Hersg), Klinische Rheumatologie, S. 374-379; Springer-Verlag, Berlin.
2. Harley JB (1998).Autoantibodies in Sjögren`s syndrome. J. Autoimmun 2: 383-394.
3. Reichlin M and Wasicek CA (1983). Clinical and biological significance of antibodies to R*o/SS-A*. Hum Pathol 14: 401-405.
4. Hendrick JP, Wolin SL, Rinke J, Lerner MR, Steitz JA (1981). Ro small cytoplasmic ribonucleoproteins are a subclass of La ribonucleoproteins: further characterization of the Ro and La small ribonucleoproteins from uninfected mammalian cells. Mol Cell Biol 1: 11381149.
5. Yoo CJ and Wolin SL (1997).The Yeast La protein is required for the 3`endonucleolytic cleavage that matures tRNA precursors. Cell 89: 393-402.
6. Lothat Thomas: Labor und Diagnose. Idikation und Bewertung von Laborbefunden fur die medizinische Diagnostil., 8. Auflage, TH Books.
7. CLSI Guideling GP44-A4: Proceduces for the Handling and Processing of Blood Specimens for Common Laboratory Tests.