Anti-Sm (IgG) ELISA

For the qualitative and quantitative determination of antibodies against Sm in serum.

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

Catalog Number: 35-SMIHU-E01
Size: 96 wells
Version: 004: 2017-08-14 - ALPCO 2.0

1. Intended Use

Sm is a solid phase enzyme immunoassay with highly purified native Smith antigen (Sm) from human eukaryotic cells (HeLa) for the quantitative and qualitative detection of antibodies against Sm in human serum. Anti-Sm antibodies recognize specific conformational epitopes only accessible on native human Sm.

2. Principle of the Assay

The term “Smith antigen“ summarizes core proteins of the U1-snRNP complex. The U1snRNP complex is a part of the splicosomal complex, that facilitates the processing of pre-mRNA to mature mRNA in the nucleus. It is a small nuclear ribonucleoprotein particle composed of uridine rich (thus U) small nuclear RNA and a set of proteins: the 70 kDa U1specific protein plus proteins A and C (all formerly summarized as RNPs) and the Sm antigen which comprises eight proteins (B,/B`, D1, D2, D3, E, F, and G). Because of its protein components Sm and RNPs the complex has been often named Sm/RNP complex.

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 20 ml	White	Yellow	5x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 X 20 ml	White	Green	50 x 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 1.5 ml	Green	Colorless	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5 ml	Red	Yellow	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	1 x 1.5 ml	Blue	Yellow	Calibrator material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5 ml	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15 ml	Blue	Blue	Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15 ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15 ml	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). Glass ware (cylinder 100100 0 ml), 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 RESEARCH USE ONLY.

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified as being irritant to eyes and skin we recommend avoiding contact with eyes and skin and to wear disposable gloves.

WARNING ! Calibrators, Controls and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed 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 the CDC or other local/national guidelines.

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

All biological source material used for some reagents of this kit (controls, standards e.g.) 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 kit controls, standards and samples 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 ALPCO.

Do not mix or substitute reagents 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: We recommend test performance at 30°C/86°F for automated systems.

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

Freshly collected serum samples are preferred. 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 x g). Blood samples should be collected in clean, dry, and empty tubes. After separation, the serum samples should be used during the first 8h, respectively stored tightly closed at 2-8°C/35-46°F up to 48h, 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 1X working sample buffer + 10 µl serum. Mix well !

Washing:
Prepare 20 ml of 1X working wash buffer 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 robot pipette.

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 1X working wash buffer into each well, wait for 20 seconds. Repeat the wash procedure twice again.

Microplates:
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

We suggest pipetting calibrators, controls and samples as follows:

For the *Quantitative* Interpretation

	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***	***…***	

For the *Qualitative* Interpretation

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

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.	+100 µl	Pipette into the designated wells as described in chapter 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. and 100 µl of each of the following: • Negative control (NC) and Positive control (PC), and • Diluted serum samples (S1, S2...)
4.	30'	Incubate for 30 minutes at 20-32°C/68-89.6°F.
5.	WASHB 3x 300µl	Wash 3x with 300 µl 1X working washing buffer (diluted 1:50).
	CONJUGATE	
6.	CONJ +100 µl	Pipette 100 µl conjugate into each well.
7.	30'	Incubate for 30 minutes at 20-32°C/68-89.6°F.
8.	WASHB 3x 300µl	Wash 3x with 300 µl 1X working washing buffer (diluted 1:50).
	SUBSTRATE	
9.	SUB +100 µl	Pipette 100 µl TMB substrate into each well.
10.	30'	Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
	STOP	

11.	STOP +100 µl	Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.	5'	Incubate 5 minutes minimum.
13.		Agitate plate carefully for 5 sec.
14.	OD_{450} - OD_{620} 450/620 nm	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 we recommend 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 – do not use for interpretation of results

Calibrators IgG	OD 450/620 nm	CV % (Variation)
0 U/ml	0.015	1.2
3 U/ml	0.138	0.1
10 U/ml	0.327	1.7
30 U/ml	0.651	2.3
100 U/ml	1.264	2.9
300 U/ml	2.081	1.4

Example of calculation – do not use for interpretation of results

Sample	Replicate (OD)	Mean (OD)	Result (U/ml)
S 01	0.594/0.598	0.596	26.6
S 02	0.878/0.854	0.866	49.3

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.

In case that the values of the controls do not meet the specified criteria, the test is invalid and must 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 we recommend 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:	2.82 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 test results obtained with this kit 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 samples 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	7.51	8.7%	7.51	20.6%	8.11	17.8%
Sample 2	20.32	11.2%	20.32	12.2%	20.57	10.7%
Sample 3	36.10	7.1%	36.10	8.3%	35.59	8.5%
Sample 4	77.31	6.0%	77.31	7.6%	77.84	5.5%
Sample 5	185.21	5.7%	185.21	7.7%	187.14	5.2%

10.2 Sensitivity and Specificity

Analytical Sensitivity

The analytical sensitivity has been assessed by multiple analysis of sample buffer and low positive samples and calculating the limit of detection. For this kit a LoD of 2.82 U/ml has been determined.

10.3 Linearity

Three sera covering the whole test 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 this assay.

10.4 Calibration

This kit is calibrated against reference sera from the CDC (Centers for Disease Control and Prevention) Atlanta. The results are expressed in U/ml.

11. Disposal

Please observe the relevant statutory requirements.

12 Literature

Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 25: 1271.

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