CE

Smith (Sm) Antibodies ELISA Kit

Semi-quantitative/Qualitative Test for Sm IgG

Product code GD63

96 tests

For *in vitro* diagnostic use

100108

1. Intended use

The Sm ELISA is a rapid method for the detection of Sm IgG. It is intended as an aid to diagnosis of systemic rheumatic diseases. The components of the kit are for *in vitro* diagnostic use only.

2. Explanation of the Test

Autoantibodies against extractable nuclear antigens (ENAs) occur in a large number of patients with systemic rheumatic diseases. These diseases are characterised by the presence of one or more ENA autoantibody.

Autoantibody specificity	Major disease association	% Incidence
SSA/Ro	Sjögren's syndrome	40-70
	SLE	25-30
	Congenital heart block	65 (mothers)
SSB/La	Sjögren's syndrome	50-60
	SLE	15
	Systemic sclerosis	*
	Neonatal lupus	*
Sm	SLE	25-40
Sm/RNP	MCTD	95-100
	SLE	25-40
Scl-70	Scleroderma	20-28
	CREST	*
	Primary Raynaud's	*
Jo-1	Dermatomyositis	18-36

3. Principle of the test

Diluted serum samples are incubated with purified Sm antigen immobilised on microtitre wells. After washing away unbound serum components, rabbit anti-human IgG conjugated to horseradish peroxidase is added to the wells and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of Stop Solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the standards, controls and samples are measured using a microplate reader at 450nm. Optical density is directly proportional to antibody activity in the sample.

4. Materials included in the kit

- **Microplate:** 96 wells in 12 X 8 break-apart strips, pre-coated with purified Sm antigen, with holder in a foil bag with desiccant
- **Reagent 1**: **Sample Diluent** 10mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 50ml, (blue), ready to use
- **Reagent 2: Wash Buffer** 100mM Tris-buffered saline with detergent, pH 7.2, 100 ml, **concentrate** (x10)
- **Reagent 3: Conjugate** rabbit anti-human IgG conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12 ml, (red), ready to use
- **Reagent 4: TMB Substrate** aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- **Reagent 5: Stop Solution** 0.25M sulphuric acid, 12 ml, ready to use
- **Standard:** 10 U/ml, 1ml of 10mM Tris-buffered saline containing human serum IgG antibodies to Sm, (yellow), ready to use
- **Positive Control:** 1ml of 10mM Tris-buffered saline containing human serum antibodies to Sm, (red), ready to use
- **Negative Control:** 1ml of 10mM Tris-buffered saline containing normal human serum, (green), ready to use
- **Instructions for use**

5. Other equipment required

1. Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 10µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable automated system may be used.
2. Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

6. Precautions

6.1 Safety Precautions

1. All reagents in this kit are for *in vitro* diagnostic use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. All human source material used in the preparation of the 10 U/ml standard and controls for this product have been tested and found negative for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the TMB Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local legislation.

6.2 Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 – 8°C after use.
3. Include the Positive and Negative Control in every test run to monitor for reagent stability and correct assay performance.
4. Strictly observe the indicated incubation times and temperature.
5. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
6. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent.
7. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
8. Do not allow microwells to dry between incubation steps.
9. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
10. Avoid direct sunlight and exposure to heat sources during all incubation steps.
11. Replace colour-coded caps on their correct vials to avoid cross-contamination
12. It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

7. Shelf life and storage conditions

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On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer has a shelf life of 3 months if stored in a closed bottle at 2 – 8°C.

8. Specimen collection and storage

Serum or plasma samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

9. Preparation of reagents

1. Dilute the Wash Buffer (**Reagent 2)** 1: 9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water.

10. Assay Procedure

1. Dilute patient samples 1:100 in diluted Sample Diluent (e.g. 5 µl serum plus 0.5 ml diluent).
2. Assemble the number of strips required for the assay.
3. For semi-quantitative assays, dispense 100 µl of sample diluent as the 0U/ml and 100 µl of the 10 U/ml standard, the Negative and Positive Controls and the diluted patient samples into appropriate wells.

   For qualitative assays, dispense only the 10 U/ml Standard together with controls and samples.
4. Incubate for **30** minutes at room temperature.
5. After 30 minutes, decant or aspirate the well contents and wash the wells 3 times using automated washing or the manual wash procedure (see below). Careful washing is the key to good results. **Do not allow the wells to dry out.**

   Manual Wash Procedure:
   Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.
6. Dispense 100µl of Conjugate (**Reagent 3)** into each well. Incubate the wells for **15** minutes at room temperature.
7. After 15 minutes, discard the well contents and carefully wash the wells 4 times with Wash Buffer. Ensure that the wells are empty but do not allow to dry out.
8. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate (**Reagent 4)** into each well. Incubate the plate for **15** minutes.
9. Add 100µl of Stop Solution (**Reagent 5)** to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.
10. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

11. Quality control

Quality control data is supplied on the lot-specific QC certificate included in the kit.

Controls are intended to monitor for substantial reagent failure.

Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

12. Interpretation of Results

For semi-quantitative results, plot the optical density of the 0 U/ml and 10 U/ml standards against concentration and draw a straight line to join the points. Read the unknowns off this line. The following table gives the expected values for samples that are normal, borderline and positive.

Sample	Concentration
Normal	< 10 U/ml
Borderline	10 – 15 U/ml
Positive	> 15 U/ml

For qualitative results, compare the optical densities of samples with that of the 10 U/ml standard.

Normal: OD sample < OD 10 U/ml standard
Positive OD sample >/= OD 10 U/ml standard

13. Limitations of the Procedure

Results must be interpreted in conjunction with other clinical information relating to each patient.

14. Performance Characteristics

The kit was compared to an established commercially available ELISA kit and the following data was obtained for 81 samples.

	Reference Sm IgG ELISA kit		
	+	-	+/-
Genesis +	43	0	0
Genesis -	0	36	0
Genesis +/-	0	0	2

15. Reproducibility

Within assay imprecision <10%

Between assay imprecision < 12%

Method Summary

- Dilute sera 1:100 with Sample Diluent **(Reagent 1)**
- Dispense sample diluent and/or the 10 U/ml Standard as required, the Positive and Negative Controls and the diluted sample into the microplate wells
- Incubate for **30** minutes at room temperature.
- Wash the wells three times
- Dispense 100µl of Conjugate **(Reagent 3)** into each well
- Incubate at room temperature for **15** minutes
- Wash the wells four times
- Add 100µl of TMB Substrate **(Reagent 4)** to each well
- Incubate at room temperature for **15** minutes
- Add 100µl Stop Solution **(Reagent 5)** to each well
- Read the optical density at 450nm (single wavelength) or 450/620nm (dual wavelength).

Further reading

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