Sm/RNP Test System

REF 2Z2841G/SM2Z2841G

IVD

CE Rx Only

INTENDED USE

The ZEUS ELISA Sm/RNP Test System is a semi-quantitative immunoassay for the detection of IgG antibodies to Sm/RNP in human sera. When performed according to these instructions, the results of this assay may aid in the diagnosis and treatment of autoimmune connective tissue disorders. This device is for *In Vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

In recent years, it has become clear that autoantibodies to a number of nuclear constituents have proven to be useful in the diagnosis of various connective tissue diseases. The Jo-1 autoantibody is one of a family of characteristic autoantibodies seen in myositis patients (19). Scientists also find them specifically in patients with myositis, and associate them with a high incidence of accompanying interstitial lung disease (10). Doctors consider antibodies directed against the Sm marker a diagnostic criterion for SLE due to high specificity for patients with SLE (1, 2). The presence of high level RNP antibodies alone are considered diagnostic of mixed connective tissue disease (MCTD) and are usually associated with a more benign disease course (3), while patients with low levels of RNP antibodies, together with other autoantibodies, may be observed in the serum of patients with progressive systemic sclerosis, Sjögren's Syndrome, and rheumatoid arthritis. The presence of RNP antibodies in the serum of SLE patients is usually associated with a lower incidence of renal involvement and a more benign disease course. To the contrary, patients with Sm antibodies experience a higher frequency of renal and central nervous system complications (4). Studies have observed autoantibodies directed against SSA and SSB in patients with SLE (5-6) and Sjögren's disease (7-9). SSA antibodies are frequently present in the serum of ANA negative SLE patients, such as subacute cutaneous lupus erythematosus (12), a lupus-like syndrome associated with a homozygous C2 deficiency (13), and in a subset of patients who lack anti-dsDNA antibodies (11). Scl-70 antibodies are highly specific for scleroderma (11). Research also observes these antibodies in a minority of SLE patients. Scl-70 positive scleroderma patients tend to have a more severe disease course, more internal organ involvement, and diffuse rather than limited skin involvement (14). Scientists rarely find Scl-70 antibodies in other autoimmune diseases, and thus, their detection in a patient with the recent onset of Raynaud's phenomenon is highly significant (15). The following table summarizes the various autoantibodies noted above with respect to disease association (16):

Antibody	Disease State	Relative Frequency of Antibody Detection %
Anti-Jo-1	Myositis	25 - 44% (19)
Anti-Sm	SLE	30*
Anti-RNP	MCTD,SLE	100** and >40, respectively
Anti-SSA (Ro)	SLE, Sjögren's	15 and 30 - 40, respectively
Anti-SSB (La)	SLE, Sjögren's	15 and 60 - 70, respectively
Anti-Scl-70	Systemic sclerosis	20 - 28*
* Highly Specific		
* *Highly specific when present alone at high titer		

The relative frequency of these autoantibodies in association with SLE and other connective tissue diseases either singularly, or as multiple autoantibodies, requires an autoantibody profile assessment of each patient's serum in order to obtain the highest degree of clinical relevance in the laboratory workup of these types of patients. Until recently, testing of autoantibodies occurred individually by indirect immunofluorescence, Ouchterlony gel diffusion, hemagglutination, radioimmunoassay, or enzyme-linked immunosorbent assay (ELISA). The exact etiology of autoimmune diseases is unknown, and the specific role played by autoantibodies in the onset of various autoimmune connective tissue diseases is obscure.

PRINCIPLE OF THE ASSAY

The ZEUS ELISA Sm/RNP Test System is designed to detect IgG class antibodies to Sm/RNP in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with Sm/RNP antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG is added to the wells and the plate is incubated. The Conjugate will react with the specific antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator, and SAVe Diluent®.**

PLATE		1. Plate: 96 wells configured in twelve, 1x8-well, strips coated with inactivated antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ		2. Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). One, 15mL, white-capped bottle. Ready to use.
CONTROL	+	3. Positive Control (Human Serum): One, 0.35mL, red-capped vial.
CAL		4. Calibrator (Human Serum): One, 0.5mL, blue-capped vial.
CONTROL	-	5. Negative Control (Human Serum): One, 0.35mL, green-capped vial.
DIL	SPE	6. SAVe Diluent®: One, 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.**
SOLN	TMB	7. TMB: One, 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN	STOP	8. Stop Solution: One, 15mL, red-capped, bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASHBUF	10X	9. Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 100mL, clear-capped, bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

NOTES:

1. **The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer. SAVe Diluent® may be used interchangeably with any ZEUS ELISA Test System utilizing Product No. 005CC.**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (20).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The SAVe Diluent®, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite).

STORAGE CONDITIONS

2°C – 8°C	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening - strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, SAVe Diluent®
2°C – 25°C	Stop Solution: 2 - 25°C Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days. Wash Buffer (10X): 2 - 25°C

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (17, 18). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and

give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (21).

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 - 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Negative Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Positive Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent®) of the Negative Control, Calibrator, Positive Control, and each patient serum. **NOTE: The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the diluent.**
4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of SAVe Diluent® to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
6. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
   a. **Manual Wash Procedure**:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps 1. and 2. for a total of 5 washes.
      4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.
   b. **Automated Wash Procedure**:
      If using an automated microwell wash system, set the dispensing volume to 300 - 350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
8. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and same order as the specimens.
9. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. → *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. → *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. → *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

	OD Range
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	≥0.500

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
   c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay Cutoff.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

The Calibrator within this Test System has been assigned both a Correction Factor for the generation of Index Values and a Calibrator Value for the generation of Unit Values. Based upon testing of normal and disease-state specimens, a maximum normal Unit Value has been determined by the manufacturer and correlated to the Calibrator.

1. **Calculations:**
   a. *Correction Factor:* The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.
   b. *Cutoff OD Value:* To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above.
   (CF x Mean OD of Calibrator = Cutoff OD Value)
   c. *Index Values/OD Ratios:* Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.

   Example:
   Mean OD of Calibrator = 0.793
   Correction Factor (CF) = 0.25
   Cutoff OD = 0.793 x 0.25 = 0.198
   Unknown Specimen OD = 0.432
   Specimen Index Value/OD Ratio = 0.432/0.198 = 2.18

   d. Conversion of Optical Density to Autoantibody Units (AAU)/mL: The conversion of OD to Unit Value (AAU/mL) can be represented by the following equation:
   Test Specimen AAU/mL = (A x B) / C Where: AAU/mL = Unknown Unit Value to be determined; A = OD of the test specimen in question; B = Unit Value of the Positive Calibrator (AAU/mL) & C = The mean OD of the Calibrator.

   Example: Test Specimen OD = 0.946
   Calibrator OD = 0.435
   Calibrator Unit Value = 155 AAU/mL
   Test Specimen AAU/mL = (0.946 x 155) / 0.435
   Test Specimen = 337 AAU/mL

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

	Unit Values	Index Value/OD Ratio
Negative Specimens	< 150 AAU/mL	≤0.90
Equivocal Specimens	150 to 180 AAU/mL	0.91 to 1.09
Positive Specimens	> 180 AAU/mL	≥1.10

Retest specimens with OD Ratio Values in the equivocal range (0.91 – 1.09) in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimens using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

LIMITATIONS OF THE ASSAY

1. Do not make a diagnosis solely on the basis of any of the ZEUS ELISA Sm/RNP Test System test results.
2. Interpret test results in conjunction with the clinical evaluation and the results of other diagnostic procedures.

EXPECTED RESULTS

The expected value for a normal patient is a negative result. The number of reactives, and the degree of reactivity is dependent upon parameters such as population type being tested, treatment, etc. Each laboratory should establish their own expected values based upon the specimens typically being tested. With respect to disease-state and percent reactivity, the table in the Significance And Background section of this Package Insert shows the relative frequency of autoantibody activity for various rheumatic disorders.

PERFORMANCE CHARACTERISTICS

1. **Comparative Study:**
   A comparative study was performed to demonstrate the equivalence of ZEUS ELISA Sm/RNP Test System to other commercially available autoantibody ELISA test systems, using 337 serum specimens; 152 normal donor samples from the northeastern and southeastern United States, and 185 disease-state repository samples previously characterized with respect to autoantibody activity. The results of the investigation have been summarized in Tables 1 and 2 below.

Table 1: Relative Sensitivity, Disease-State Specimens

ZEUS ELISA Reactives	Commercial ELISA Reactives	Discrepant Samples	Reactives After Resolution of Discrepants	Sensitivity
46	58	11	50	46/50 = 92.0%

Table 2: Relative Specificity; Normal Donor Specimens

ZEUS ELISA Non-Reactives	Commercial ELISA Reactives	Discrepant Samples	Non-reactives After Resolution of Discrepants	Specificity
141	144	3	144	141/144 = 97.9%

2. **Reproducibility:**
   A reproducibility study was conducted to assess the intra-assay and inter-assay variability of the test system using a strong positive, a low positive, and a negative sample. Samples were tested eleven times on each of three days. The mean unit value, the standard deviation, and the percent CV were calculated for each sample. The results of this study are depicted below:

Table 3: Reproducibility for ZEUS ELISA Sm/RNP Test System

	Intra-assay Reproducibility									Inter-Assay Reproducibility All Days Combined		
	Day 1			Day 2			Day 3					
Specimen	Mean	Std D	% CV	Mean	Std D	% CV	Mean	Std D	% CV	Mean	Std D	% CV
High Positive	535	73	14	426	73	17	608	76	12	532	97	18
Low Positive	184	34	18	246	34	14	216	29	13	216	42	19
Negative	26	4	N/A	29	9	N/A	22	6	N/A	26	7	N/A

3. **Cross Reactivity:**
   Specimens negative for ANA by HEp-2 IFA and positive for IgG antibody to various antigens such as EBV-VCA, EBNA, HSV-1, HSV-2, CMV, Rubella, and/or Toxo, were tested for potential cross reactivity using the ZEUS ELISA Sm/RNP Test System. All specimens tested were negative on the ELISA, indicating that the potential for cross reactivity with such antibodies is not likely, and therefore should not interfere with the results obtained.

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