AccuDiag™
ENA Profile-6
(SM/RNP, Sm, Jo-1, Scl-70, SS-A, SS-B)
ELISA Kit

REF 2506-2

IVD See External Label 2°C-8°C 96 Tests

ENA Profile ELISA	
Principle	Indirect ELISA
Detection	Semi-Quantitative
Sample	10 µL serum/plasma
Incubation Time	65 minutes
Sensitivity	96%
Specificity	100%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation, Inc. ENA Profile-6 ELISA test system is a semi-quantitative immunoassay for the detection of IgG antibodies to Jo-1, Sm, Sm/RNP, SSA (Ro), SSB (La), and Scl-70 in human sera. When performed according to these instructions, the results of this autoantibody profile may aid in the diagnosis and treatment of autoimmune connective tissue disorders. This device is for in vitro diagnostic use.

SIGNIFICANCE AND SUMMARY

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 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. The Diagnostic Automation, Inc. ELISA ENA Profile-6 Test System offers an efficient test procedure for the laboratory workup of patients with various connective tissue diseases using the association and frequency of detection of these antibodies.

ASSAY PRINCIPLE

The DAI ENA Profile-6 ELISA test system is designed to detect IgG class antibody to different autoantigens in human sera. Wells of plastic microwell strips are sensitized by passive adsorption with immobilized antigens. The test procedure involves three incubations 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 antibodies 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.

SPECIMEN COLLECTION & PREPARATION

1. It is recommended that specimen collection be carried out 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, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (17, 18). No anticoagulants or preservatives should be added. 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 and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that 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).

MATERIALS AND COMPONENTS

Materials provided with the test kit

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

1. **Plate:** 96 wells configured in twelve, 1x 8-well, strips coated with inactivated antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant
2. **Conjugate:** Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). Ready to use. One, 15 mL, white- capped bottle.
3. **Positive Control (Human Serum):** One, 0.35 mL, red-cap.
4. **Calibrator (Human Serum):** Two, 1.0mL, vial with a red crimp cap (lyophilized). Preservatives added. Reconstitute the contents of the vial with 1.0 mL distilled or deionized water. After reconstitution, the Calibrator is ready to use. **Do not dilute additionally before use.**
5. **Negative Control (Human Serum):** One, 0.35mL, green-cap.
6. **Sample Diluent:** One, 30mL, green-cap, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Ready to use. **Note: The Sample Diluent will change color when combined with serum.**
7. **TMB:** One, 15 mL, amber-capped, amber bottle containing 3, 3', 5, 5' – tetramethylbenzidine (TMB). Ready to use.
8. **Stop Solution:** One, 15 mL, red-capped, bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
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.**

The following components are not kit lot number dependent and may be used interchangeably with the ELISA assays: TMB, Stop Solution, and Wash Buffer.

Note: Kit also contains

1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

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 to 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. (Example: 10% household bleach - 0.5% sodium hypochlorite.)

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Each specimen will require one, 1x8 profile antigen strip. Include the Positive Control, Negative Control, and Calibrator each time the assay is run. 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.
3. Prepare a 1:21 dilution of the Positive and Negative Controls, and each patient serum.
   a. Add 50µL of each sample, the Positive Control, and the Negative Control to separate tubes. Add 1000µL of Sample Diluent to each tube. Mix the contents of each tube thoroughly. The Sample Diluent will undergo a color change confirming that the specimen has been combined with the diluent.
   b. Add 100µL of Sample Diluent to every well in row A.
   c. Using a multichannel pipette, load the Controls, Calibrator, and patient specimens as outlined below. Load 100µL per well. The reconstituted Calibrator is ready to load, **do not dilute.**

		Column Number:					
Antigen:	**Row:**	**1**	**2**	**3**	**4**	**5**	**ETC.**
Ag Mixture	**A**	Dil	Dil	Dil	Dil	Dil	
Jo-1	**B**	NC	PC	Cal	Test 1	Test 2	
Sm	**C**	NC	PC	Cal	Test 1	Test 2	
Sm/RNP	**D**	NC	PC	Cal	Test 1	Test 2	
SSA	**E**	NC	PC	Cal	Test 1	Test 2	
SSB	**F**	NC	PC	Cal	Test 1	Test 2	
Scl-70	**G**	NC	PC	Cal	Test 1	Test 2	
Control Ag	**H**	NC	PC	Cal	Test 1	Test 2	

4. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
5. 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.

6. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and same order as the specimens.
7. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
8. Wash the microwells by following the procedure as described in step 5.
9. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
10. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
11. 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.
12. 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.

RESULTS

1. **Calculations:**

a. **Determining Autoantigen-Specific Optical Density (OD):**
Controls, calibrator, and test specimens are all loaded on the control antigen well (Row H). The control antigen well is prepared by coating and blocking the plastic with solutions which **do not** contain any autoantigens. Therefore, the control antigen well provides measurement of **non-specific absorbency** in each sample. Autoantigen-specific absorbency may be represented as the difference in optical density between the antigen coated well and the control antigen well.

Example:
Test Specimen A, Control Well OD (Row H) = 0.175
Test Specimen A, SSB Well OD (Row F) = 1.563
SSB Specific OD = 1.563 - 0.175 = 1.388

Using the respective control antigen well for each calibrator, control, and sample, determine the autoantigen-specific OD for each autoantigen. Do not subtract the control antigen OD from the conjugate control well OD.

b. **Calibrator:**
Based upon testing of normal and disease-state specimens, a maximum normal autoantibody unit (AAU) value has been determined by Diagnostic Automation, Inc. and correlated to the calibrator. The calibrator will allow you to determine the unit value of test samples for each of the autoantigens, and to correct for slight day-to-day variations in test results. The unit value (CV) is determined for each autoantibody antigen for each lot of kit components and is printed on the Component List.

c. **Conversion of Optical Density to AAU/mL:** The conversion of autoantigen-specific 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 test specimen in question.
B = Unit value of calibrator for autoantigen in question (AAU/mL).
C = OD of calibrator.
Example: Test specimen specific OD for SSA = 0.946
Calibrator specific OD for SSA = 0.435
Calibrator unit value for SSA = 155 AAU/mL
Test Specimen AAU/mL = (0.946 x 155)/0.435
Test Specimen = 337 AAU/mL for anti-SSA

2. **Interpretation of Results:**
Using 152 normal healthy donor specimens, and 185 disease-state specimens, DAI has established the following guidelines for the interpretation of patient results:

<150 AAU/mL - Negative
150 - 180 AAU/mL - Equivocal
>180 AAU/mL - Positive

Use the above guidelines when evaluating or interpreting patient specimens. Re-test specimens with OD ratio values in the equivocal range (0.91 – 1.09) in duplicate. Report any two of the three results which agree. Test repeatedly equivocal specimens using an alternate serologic procedure and/or re-evaluate by drawing another sample one to three weeks later. Elevated autoantibody levels to any of the profile autoantigens may be indicative of a specific rheumatic disorder. The Significance and Background section of this Package Insert describes some of the more common diseases associated with elevated autoantibody levels. **NOTE: When interpreting the anti-Sm/RNP result to determine potential anti-RNP (only) activity, one must consider the anti-Sm and the anti-Sm/RNP result simultaneously.** For example, below are three possible scenarios:

a. Anti-Sm result = 80, and anti-Sm/RNP result = 986 AAU/mL. Patient expresses a significant amount of anti-RNP.
b. Anti-Sm result = 493 AAU/mL, and anti-Sm/RNP result = 1139 AAU/mL. Patient expresses significant amounts of both autoantibodies.
c. Anti-Sm result = 37 AAU/mL, and anti-Sm/RNP result = 63 AAU/mL. Patient is negative for both autoantibodies.

QUALITY CONTROL

1. When performing the assay, test the Positive Control, Negative Control, and Calibrator. For each profile strip, it is necessary to include a Diluent Blank (Row A), and a Control Antigen Blank (Row H). The Diluent Blank measures the nonspecific interaction between the Conjugate and the autoantigens. The Control Antigen well provides a measurement of non-specific interaction between patient antibody and a Control Antigen.
2. Refer to the included Component List. This sheet describes the lot specific specifications for the Calibrator. If the Calibrator is out of range, the results are considered invalid, and patient results may not be reported.
3. The Positive and Negative Controls must meet the following specifications:
   a. Positive Control must be >180 AAU/mL
   b. Negative Control must be <150 AAU/mL
   c. Positive Control/Negative Control must be ≥2.00
4. If the assay Controls do not meet the above specifications, then the assay is considered invalid. Do not report the patient results.
5. Calculate the mean of the Conjugate Control wells (Row A). This value should be less than 0.200. A high mean OD for the Conjugate control could indicate inadequate washing, poor water quality, or contaminated reagents. Variability from well-to-well indicates poor pipetting and/or inconsistency of washing, well-to-well.

PERFORMANCE CHARACTERISTICS

Comparative Study

A comparative study was performed to demonstrate the equivalence of the Diagnostic Automation, Inc. ELISA ENA Profile-6 Test System to several 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. *The total population tested for anti-Jo-1 was 126; 64 normal donor samples, and 62 of the disease-state repository samples.

TABLE 1 Relative Sensitivity; Disease-State Specimens

Autoantigen	DAI ELISA Reactives	Commercial ELISA Reactives	Discrepants	Reactives After Discrepant Resolution	Sensitivity
Jo-1	8	8	0	8	8/8 = 100.0%
Sm	13	16	3	13	13/13 =100.0%
Sm/RNP	46	58	11	50	46/50 = 92.0%
SSA	56	74	18	57	56/57 = 98.2%
SSB	28	34	6	29	28/29 = 96.6%
Scl-70	8	17	9	8	8/8 = 100.0%

Table 2: Relative Specificity, Normal Donor Specimens

Autoantigen	DAI ELISA Non-Reactives	Commercial ELISA Non-Reactives	Discrepants	Non-Reactives After Discrepant Resolution	Specificity
Jo-1	64	64	0	64	64/64 =100.0%
Sm	136	137	1	137	136/137 =99.3%
Sm/RNP	141	144	3	144	141/144=97.9%
SSA	146	146	0	146	146/146=100.0%
SSB	147	147	0	147	147/147=100.0%
Scl-70	151	151	0	151	151/151=100.0%

Reproducibility

To assess the intra-assay and inter-assay variability of the test procedure, a strong positive, a low positive, and a negative sample for all of the autoantigens 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 in Tables 3 - 6 below:

Table 3: Intra-Assay Reproducibility, "High Positive" Specimen: DAI. ENA Profile-6 IgG ELISA

	Day 1			Day 2			Day 3		
Antigen	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
Jo-1	459	15	3	391	22	6	385	18	5
Sm	576	71	12	690	71	10	702	29	4
Sm/RNP	535	73	14	426	73	17	608	76	12
SSA	818	62	7	652	68	10	779	52	7
SSB	1022	120	12	881	65	7	987	67	7
Scl-70	669	95	14	626	65	10	726	93	3

Table 4: Intra-Assay Reproducibility, "Low Positive Specimen; DAI. ENA Profile-6 IgG ELISA

	Day 1			Day 2			Day 3		
Antigen	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
Jo-1	232	11	5	189	9	4	189	8	4
Sm	460	43	9	587	52	9	392	28	7
Sm/RNP	184	34	18	246	34	14	216	29	13
SSA	199	26	13	231	38	17	189	22	12
SSB	178	29	16	167	20	12	210	25	12
Scl-70	231	21	9	214	10	5	270	21	8

Table 5: Intra-Assay Reproducibility, Negative Specimen; DAI. ENA Profile-6 IgG ELISA

	Day 1			Day 2			Day 3		
Antigen	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
Jo-1	5	2	N/A	5	1	N/A	4	1	N/A
Sm	12	3	N/A	8	3	N/A	7	1	N/A
Sm/RNP	26	4	N/A	29	9	N/A	22	6	N/A
SSA	27	4	N/A	14	6	N/A	13	5	N/A
SSB	2	2	N/A	1	1	N/A	1	1	N/A
Scl-70	5	2	N/A	5	3	N/A	3	2	N/A

Table 6: Intra-Assay Reproducibility; DAI. ENA Profile-6 ENA ELISA

	Day 1			Day 2			Day 3		
Antigen	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
Jo-1	412	38	9	203	23	11	5	2	N/A
Sm	656	85	13	479	93	19	9	3	N/A
Sm/RNP	532	97	18	216	42	19	26	7	N/A
SSA	750	95	13	207	35	17	18	9	N/A
SSB	963	108	11	185	32	17	1	1	N/A
Scl-70	674	97	14	238	30	13	5	2	N/A

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 DAI. ENA Profile-6 ELISA 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.

LIMITATIONS OF THE ASSAY

1. A diagnosis should not be made solely on the basis of the ENA Profile-6 ELISA test results.
2. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.

EXPECTED RANGES OF VALUES

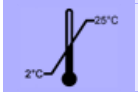
The expected value for a normal patient is a negative result. The number of reactives, and the degree of reactivity is dependent upon parameters such as population type being tested, treatment, etc. Each laboratory should establish their own expected values based upon the specimens typically being tested. With respect to disease-state and percent reactivity, Table 1 in the Significance and Background section of this package insert shows the relative frequency of autoantibody activity for various rheumatic disorders.

PRECAUTIONS

1. For In Vitro Diagnostic Use
2. Normal precautions exercised in handling laboratory reagents should be followed. 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, the strips should be considered potentially biohazardous materials and handled 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 Sample 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. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of 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. Reagents from other sources or manufacturers should not be used.
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: Liquid waste at acid pH should be neutralized before adding to 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.
27. Fully reconstitute the Calibrator prior to performing the assay. Improper or inadequate reconstitution will produce erroneous results.

STORAGE CONDITIONS

	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, Sample Diluent
	Unused reconstituted Calibrator, up to 30 days
	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

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

Date Adopted	**2023-09**
Brand Name	**AccuDiag™**
REF 2506-2	**AccuDiag™ - ENA Profile-6 ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu**
Revision Date: 09/15/2020	