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IVD



See external label



2°C-8°C



Σ=96 tests

REF

#2571

**HISTONE**For *in vitro* diagnostic use.**Catalog No. 2571****INTENDED USE**

The Diagnostic Automation, Inc. (DAI) Histone Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the detection and semi-quantitation of antibodies to histone in human sera. The assay is to be used to detect antibodies in a single serum specimen. The results of the assay are to be used as an aid to the diagnosis of drug induced lupus. For *in vitro* diagnostic use.

**INTRODUCTION**

Systemic autoimmune disease is characterized by the presence of circulating autoantibodies directed to wide variety of cellular antigens (1,2,3). Systemic lupus erythematosus (SLE), commonly referred to as Lupus is best known of these diseases. Other possible connective tissue diseases include mixed connective tissue disease (MCTD), Sjogren syndrome, scleroderma, and polymyositis/dermatomyositis. The majority can be diagnosed by clinical presentation and their antibody profiles to the various antigens involved, which include dsDNA, SM, RNP, Ro, La, Scl-70, Jo-1, and Histones. Therefore, immunoassays for autoantibodies are useful for diagnostic and prognostic evaluations of autoimmune disease (1,2,3).

Histones are a group of small basic proteins that are complexed with DNA. Antibodies to histone are present in approximately 90% of patients with drug induced lupus (DIL). They are also present in approximately 30% of patients with idiopathic lupus (SLE). Patients with DIL usually have antibody to just histone, while SLE patients usually have antibodies to DNA or a variety of ENA's (1,2,3).

Classically, antibodies to autoimmune antigens are detected by double immunodiffusion. However, the test is lengthy and suffers weak sensitivity. Enzyme-Linked Immunosorbent Assays (ELISAs) combine greater sensitivity with ease of use. Many ELISAs have been developed and validated for detecting autoantibodies to various antigens (4,5,6,7).

**PRINCIPLE OF THE TEST**

The DAI Histone test is an Enzyme-Linked Immunosorbent Assay to detect IgG, IgM, and IgA antibodies to histone antigens. Purified histone antigens are attached to a solid phase microassay well. Diluted test sera are added to each well. If the antibodies are present that recognize the antigen, antigen-antibody complexes are formed. After incubation the wells are washed to remove unbound antibody. An enzyme labeled anti-human IgG, M, A, is added to each well. If antibody is present the conjugate will bind to the antigen-antibody complexes. After incubation the wells are washed to remove unbound conjugate. A substrate solution is added to each well. If enzyme is present the substrate will undergo a color change. After an incubation period the reaction is stopped and the color intensity is measured photometrically, producing an indirect measurement of specific antibody in the patient specimen.

**KIT COMPONENTS**

1. Histone antigen coated microassay plate: 96 well, provided with a strip holder and stored in a foil bag with desiccant and humidity indicator card.
2. Wash Buffer (20x concentrate): One bottle, 50 mL. Contains buffer and Tween 80.
3. Serum Diluent: One bottle, 30 mL. Contains buffer, BSA and Tween 80.
4. Conjugate: One bottle, 15 mL. Contains horseradish peroxidase conjugated anti-human IgG, IgM and IgA in a buffer.

5. Substrate: One bottle, 15 mL. Contains 3, 3', 5, 5' - tetramethylbenzidine (TMB).
6. Stop Solution: One bottle, 15 mL. Contains a H<sub>2</sub>SO<sub>4</sub> solution.
7. High Positive Control: One vial, 0.4 mL. Contains human serum with antibodies that react strongly with the antigen. Established range printed on vial label.
8. Negative Control: One vial, 0.4 mL. Contains human sera with antibodies that do not react with the antigen. Established range printed on vial label.
9. Low Positive Control: One vial, 0.4 mL. Contains human serum with antibodies that react weakly with the antigen. Established range printed on vial label.
10. Calibrator: One vial, 0.4 mL. Contains human serum with antibodies that react with the antigen used to calibrate the assay. Kit specific Correction Factor printed on vial label.

**The following components are not kit lot# dependent and may be used interchangeably within the DAI ELISA autoimmune kits: Serum Diluent Type III, Chromogen/Substrate Solution Type I, Wash Buffer Type II, and Stop Solution. Please check that the appropriate DAI reagent type (Type I, Type II, etc...) is used for the assay.**

## REAGENT STORAGE CONDITION

1. All kit components that are stored at their recommended storage conditions are stable until the expiration date on their label. Do not use past their expiration date.
2. Antigen coated wells. Unused strips should be immediately resealed in the foil bags with desiccant and humidity indicator card and returned to storage at 2-8° C. If the bag is resealed with tape the wells are stable for 30 days. If the bag is resealed with a heat sealer the wells are stable until their expiration.
3. All other reagents are stored at 2-8° C in their original containers.
4. Store 1X (diluted) Wash Buffer at room temperature (21° to 25° C) for up to 5 days, or 1 week between 2° and 8° C.

## PRECAUTIONS

1. Each donor unit used in the preparation of the Calibrator and Controls was tested by an FDA approved method for the presence of the antibody to HIV-1 as well as for hepatitis B surface antigen and found to be negative. Because no test method can offer complete assurance that human immunodeficiency virus (HIV-1), hepatitis B virus, or other infectious agents are absent, these specimen/reagents as well as patient samples, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control / National Institute of Health manual "Biosafety in Microbiology and Biomedical Laboratories", 1993 and FDA labeling guidelines for *in vitro* diagnostic reagent manufactures, sec. 1985. (8).
2. Certain reagents in this kit contain sodium azide for use as a preservative. Azides may react with lead and copper plumbing to form explosive azide compounds. When disposing of reagents, flush with copious quantities of water to minimize azide build up.
3. This product is for IN VITRO DIAGNOSTIC USE only.
4. Reagents contain preservatives which may be toxic if ingested.
5. Do not pipette by mouth. Avoid contact of reagents and patient specimens with skin or mucous membranes.
6. Do not allow the stop solution to contact skin or eyes. If contact occurs, immediately flush with copious quantities of water.
7. Avoid splashing or generation of aerosols.
8. Do not use heat inactivated sera.
9. Do not mix or interchange reagents between lots of kits or from other manufacturer.
10. Do not dilute or adulterate kit reagents.
11. Do not cross contaminate reagents or specimens.
12. Do not use TMB Substrate solution if it has begun to turn blue.
13. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
14. Do not vary reagent and incubation temperatures above or below room temperature ( 21 - 25° C ).
15. Washing is important. Improperly washed wells will give erroneous results. Do not allow the well to dry out between incubations.

## SPECIMEN COLLECTION

1. Aseptically collect blood samples by venipuncture and prepare serum using accepted technique (9).
2. Serum containing visible particulate matter can be spun down utilizing slow speed centrifugation.
3. Sera may be stored up to five days at 2-8° C. If a further delay in testing is needed store frozen at -20 to -70° C in a non-defrosting freezer. Avoid multiple freeze/thaw of patient samples.
4. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
5. Do not heat inactivate sera.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200  $\mu$ L volumes (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water, CAP Type 1 or equivalent.
9. Timer capable of measuring to an accuracy of  $\pm$  1 second.
10. Disposal basins and 0.5% sodium hypochlorite ( 50 mL bleach in 950 mL H<sub>2</sub>O).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the operators' manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

## PREPARATION OF REAGENTS

1. All reagents must be removed from refrigeration and allowed to come to room temperature ( 21 - 25° C) before use. Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer to 1 L with distilled and/or deionized H<sub>2</sub>O. Mix well.

## TEST PROCEDURE

1. Determine the number of patients to be assayed. For each assay the Calibrator should be run in duplicate. Also the High Positive Control, Low Positive Control, Negative Control, and a reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Calibrator/Control configurations.

Example Configuration:

1A	RB	2A	Patient #2
1B	NC	2B	Patient #3
1C	Cal	2C	Patient #4
1D	Cal	2D	Patient #5
1E	Cal	2E	Patient #6
1F	HPC	2F	Patient #7
1G	LPC	2G	Patient #8
1H	Patient #1	2H	Patient #9

2. For each test serum, Calibrator and Control to be assayed prepare a 1:21 serum dilution. Add 10  $\mu$ L of each serum sample to 200  $\mu$ L of Serum Diluent . Mix well.
3. To individual wells, add 100  $\mu$ L of the appropriate diluted Calibrator, Controls and patient sera. Add 100  $\mu$ L of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21-25)°C for 30 minutes  $\pm$  1 minutes.
5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300  $\mu$ L of diluted Wash Buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure two times (for a total of three (3) washes) for manual or semi-automated equipment or four times (for a total of five (5) washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid form the wells.

\*\* Important note: Regarding steps 5 and 8 – Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a

volume to completely fill each well (250-300 uL) is recommended. A total of up to five (5) washes may be necessary with automated equipment. Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.

6. Add 100 uL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21-25)°C for 30 minutes +/- 1 minutes.
8. Repeat wash as described in Step 5.
9. Repeat wash as described in Step 6.
10. Incubate each well at room temperature (21-25)°C for 15 minutes +/- 2 minutes.
11. Stop reaction by addition of 100 uL of Stop Solution (1N Sulfuric Acid) following the same order of Chromogen/Substrate (TMB) addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. Wait a minimum of 5 minutes and read. The plate may be held up to 1 hour after addition of the Stop Solution before reading.
12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600 – 650 nm. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is  $\geq 0.150$  the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

## CALCULATIONS

1. Mean Calibrator Value - Calculate the mean value for the Calibrator from the three Calibrator determinations.
2. Correction Factor - To account for day to day fluctuations in assay activity due to room temperature and timing, a correction factor is determined by DAI for each lot of kits. The correction factor is printed on the Calibrator vial.
3. Cutoff O.D. Value - The cutoff O.D. value for each assay is determined by multiplying the correction factor by the mean Calibrator value determined in step 1.
4. Index Value - Calculate an index value for each specimen by dividing the specimen O.D. value by the cutoff O.D. determined in step 3.

Example :

O.D.s obtained for Calibrator	= 0.38, 0.42, 0.40
Mean O.D. for Calibrator	= 0.40
O.D. obtained for patient sera	= 0.60
Correction factor	= 0.50
Cutoff value	= $0.50 \times 0.40 = 0.20$
Index Value	= $0.60/0.20 = 3.00$

## INTERPRETATION OF RESULTS

<u>Index Value</u>	<u>Interpretation</u>
$\leq 0.90$	Negative
0.91 - 1.09	Equivocal
$\geq 1.10$	Positive

Specimens with index values in the equivocal range should be retested. If still equivocal retest by an alternate method or test a new sample.

## QUALITY CONTROL

1. Calibrator and Controls must be run with each test run.
2. Reagent blank must be  $< 0.15$  O.D. at 450 nm.
3. The mean O.D. value for the Calibrator should be  $\geq 0.30$  at 450 nm.
4. The index values for the High, Low, and Negative Control should be in their respective ranges printed on the vials. If the control values are not within their respective ranges, the test should be considered invalid and the test should be repeated.
5. If above criteria are not met on repeat, contact DAI Technical Service.

## LIMITATIONS

1. The result of the assay should not be interpreted as being diagnostic. The results should only be used as an aid to diagnosis. The results should be interpreted in conjunction with the clinical evaluation of the patient.
2. Sera from patients with other autoimmune disease and from normal individuals may contain autoantibodies.
3. The assay should be used only with serum. Icteric, lipemic, hemolyzed, and heat inactivated serum should be avoided.
4. Index Values of  $\geq 10.00$  should be reported as greater than 10.

## EXPECTED VALUES

1. From 1-5% of apparently normal individuals may contain autoantibodies (1,2,3).
2. Antibodies to histones are present in approximately 90% of patients with drug induced lupus (DIL) and approximately 30% of patients with idiopathic lupus (SLE) (1,2,3).
3. Patients with DIL usually have antibody to just histones, while SLE patients have antibodies to DNA or a variety of ENA's (1,2,3).

## PERFORMANCE CHARACTERISTICS SENSITIVITY AND SPECIFICITY

The DAI Histone ELISA kit was evaluated relative to a commercially available ELISA test kit. Table 1 summarizes the data.

**Table 1**  
**Sensitivity and Specificity of the DAI Histone ELISA Kit**

		DAI Histone ELISA Kit			Total
		Positive ≥ 1.10	Equivocal 0.91-1.09	Negative ≤ 0.90	
Alternate	Positive > 1.10	20	1	2	23
ELISA	Equivocal 0.91-1.09	2	0	4	6
Kit	Negative < 0.90	1	1	79	81
	Total	23	2	85	110

Sera falling in the equivocal range were excluded from the following calculations

Relative Sensitivity	= 20/22	= 90.9%
Relative Specificity	= 79/80	= 98.8%
Relative Agreement	= 99/102	= 97.1%

## PRECISION

The precision of the DAI Histone ELISA kit was determined by testing seven different sera eight times each on three different assays. The data is summarized in Table 2. With proper technique the user should obtain C.V.'s of less than 20%.

**Table 2**  
**Precision Data**

Serum #	Assay 1 (n=8)			Assay 2 (n=8)			Assay 3 (n=8)			Inter Assay (n=24)		
	X	S.D.	C.V.	X	S.D.	C.V.	X	S.D.	C.V.	X	S.D.	C.V.
1	3.27	.492	15.1%	3.04	.332	10.9%	3.52	.574	16.3%	3.28	.498	15.2%
2	1.94	.305	15.7%	2.38	.330	13.9%	2.15	.222	10.3%	2.16	.332	15.4%
3	2.76	.240	8.70%	3.07	.271	8.83%	3.24	.334	10.3%	3.13	.339	11.2%
4	2.35	.388	16.5%	2.60	.347	13.3%	2.55	.389	15.3%	2.50	.375	15.0%
5	0.00	.000	0.00%	.005	0.11	220%	.010	0.19	190%	.010	.013	130%
6	0.04	.113	283%	.001	.004	400%	.026	.023	100%	.023	.066	287%

X = Mean Histone Value  
S.D. = Standard Deviation  
C.V. = Coefficient of Variation

## LINEARITY

The DAI Histone index values were determined for serial twofold dilutions of five positive sera. The index values were compared to log<sub>2</sub> of dilution by standard linear regression. The data in Table 3 indicates that the assay is semi-quantitative.

**Table 3**  
**Linearity**

<u>Serum #</u>	<u>Neat</u>	<u>1:2</u>	<u>1:4</u>	<u>1:8</u>	<u>1:16</u>	<u>r<sup>2</sup></u>
1	3.00	2.00	1.10	0.50		.998
2	2.50	1.20	0.60			.957
3	3.80	2.10	1.10	0.40		.961
4	2.88	2.06	1.31	0.63		.998
5	4.13	3.13	2.25	1.63	0.94	.990

r<sup>2</sup> = coefficient of determination. Linear regression compared Histone Value to log<sub>2</sub> of dilution.

### CROSS REACTIVE DATA

Sera containing high level of antibodies to potentially cross reactive antigens were assayed on the DAI Histone ELISA kit. The data in Table 4 indicate that antibodies to alternate autoimmune antigens do not cross react with the DAI Histone ELISA kit.

**Table 4**  
**Cross Reactive Data**

<u>Serum #</u>	<u>Antibody Specificity</u>	<u>Histone Index Value</u>	<u>Interpretation</u>
1	SM	0.01	-
2	SM	0.06	-
3	SM	0.57	-
4	RNP	0.56	-
5	RNP	0.02	-
6	RNP	0.06	-
7	Ro	0.10	-
8	Ro	0.34	-
9	Ro	0.01	-
10	La	0.07	-
11	La	0.01	-
12	La	0.20	-
13	Scl-70	0.36	-
14	Scl-70	0.28	-
15	Scl-70	0.34	-
16	Jo-1	0.22	-
17	Jo-1	0.05	-
18	Jo-1	0.04	-
19	dsDNA	0.07	-
20	dsDNA	0.32	-
21	dsDNA	0.58	-

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