Package Insert

Human Anti-Histones ELISA

Catalog Number: HTS31-K01

For Research Use Only. Not for use in diagnostic procedures.

v. 2 (10 OCT 24)

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INTENDED USE

Human Anti-Histone ELISA (IgG) is an *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit for the accurate quantitative measurement of IgG class antibodies against total histones in human serum or plasma.

INTRODUCTION

Histones are cationic proteins which associate with DNA in the nucleus of eukaryotic cells to form nucleosomes. Anti-histone antibodies occur in a number of clinical conditions, primarily in systemic lupus erythematosus (SLE) and drug-induced lupus (DIL), and in other systemic and organ specific autoimmune diseases, and certain neurological and infectious diseases. Anti-histone antibodies are found in up to 80% of SLE patients, and 95% of the cases with DIL by procainamide, hydralazine, chlorpromazine, and quinidine. Besides SLE and DIL, anti-histone antibodies are commonly seen in other rheumatic diseases, including myositis and systemic sclerosis (SSc). Therefore, anti-histone antibodies are a common biomarker for evaluating the autoimmune diseases.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

ASSAY PRINCIPLE

The determination of anti-histone antibodies is based on an indirect enzyme linked immune reaction. The microtiter plate is pre-coated with purified total histones, which bind to the anti-histone antibodies present in the standards and samples. After incubation and washing, any unbound antibodies will be removed. Then 100x anti-human IgG horseradish peroxidase (HRP) conjugates are added, which bind to the captured antihistone antibodies. After incubation and washing, any unbound conjugates will be also removed. Then substrate is catalyzed by the HRP to produce a blue color that changes to yellow after adding the stopping buffer. The density of the yellow coloration is directly proportional to the amount of captured anti-histone antibodies in the plate. The light absorbance (OD value) under 450nm wavelength of the wells is determined using a microplate reader. The antibody concentration of the unknown sample can be estimated with the provided calibrators in the kit. Since no international standard has been established for anti-histone antibodies, the standards are calibrated against AntiNuclear Factor Serum (Homogeneous) Human (NIBSC code: W1064, non-WHO reference material), and presented as relevant unit (RU) per mL. The kit offers semiquantitative and quantitative interpretation of the data, which is in the section of **DATA INTERPRETATION.**

STORAGE

The kit should be stored at 2-8°C upon receipt. Remove any unused antibody-coated strips from the microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips can be stored at 2-8°C for up to one month.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:
1. ELISA plate, covered with purified total histones for detecting human sourced antihistone
antibodies, 12 strips (8 wells/strip), sealed
2. 5xSample buffer, 12 mL
3. Calibrator 1 (10 RU/mL)
4. Calibrator 2 (50 RU/mL)
5. Calibrator 3 (300 RU/mL)
6. Positive control, human sourced
7. Negative control, human sourced
8. l0xWash buffer, 50 mL
9. lO0x anti-human lgG-HRP solution, 120 µL
10. Substrate solution, 12 mL, ready for use
11. Stopping solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

I. Pipettes or pipette tips
2. Microplate washer
3. Buffer and reagent containers
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbance at 450 nm
6. Distilled/deionized water

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1x Sample buffer

Prepare 1x Sample buffer by mixing the 5xSample buffer (20 mL) with 80 mL of distilled water or deionized water. If precipitates are observed in the 5x Sample buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1x Sample buffer may be stored at 2-8°C for up to one month.

B. Sample preparation

Vortex and centrifuge the sample tubes with a microcentrifuge at 16,000x g for 1 minute. Dilute the sample (serum or plasma) into sample buffer following the ratio of 1:101, and store under 2-8°C before further usage.

C. Calibrators, Positive control and Negative control

The calibrators and controls are diluted with sample buffer following the ratio of **1:** 101, and store under 2-8°C before further usage.

D. 1X Wash buffer

Prepare 1x Wash buffer by diluting the J0xWash buffer (50 mL) with 450 mL of .. distilled/deionized water (v/v = 1:9). If crystals are observed in the !Ox Washing buffer bottle, incubate the bottle in a 37°C water bath until the crystals is fully dissolved and further vortex the bottle for 1 minute. The **1** xWash buffer can be stored at 2-8°C for up to one month.

E. 100x anti-human lgG - HRP solution

Spin down the lO0xanti-human IgG-HRP briefly and dilute the desired amount of the antibody 1:100 with 1x Sample buffer, 100 µL of the 1x Detection antibody solution is required per well. Prepare only as much 1x Detection antibody solution as needed. Return the 100x Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

F. Substrate solution
Substrate solution is ready for use. As the solution is highly sensitive to the light, ensure the bottle is fully closed after use. The solution is clear and colorless. Dispose the solution if it turns blue.
G. Stopping solution
Stopping solution contains H2SO4, ready for use.

ASSAY PROCEDURE

It is recommended that all standards and samples be run with blank wells and in duplicate.

1. Add 100 µL of calibrator, positive control, negative control or sample dilution into each well; incubate at room temperature (around 23°C) for 30 minutes.
2. Discard the content and tap the plate on a clean paper towel to remove residual liquid in each well. Add 300 µL of 1x Wash buffer to each well and incubate for 1 minute. Discard the 1x Wash buffer and tap the plate on a clean paper towel to - remove residual wash buffer. Repeat the wash step for a total 3 washes.
Note: When the residual in the well (> 10 µL) can interfere the reaction between the reagents, leading to a lower OD value. The inadequately washing (e.g., less than 3 repeats, inadequate wash buffer or washing for a short period of time) of the plates can cause a higher OD value.
3. Add 100 µL of diluted l00x anti-human IgG-HRP solution to each well, incubate at room temperature (around 23°C) for **1** hour.
4. Wash each well 3 times as in step 2.
5. Add 100 µL of Substrate solution to each well (e.g., *5* seconds between two wells), incubate at room temperature for 10 minutes.
6. Add l 00 µL of Stopping solution to each well with the same pace as adding the substrate (e.g., *5* seconds between two wells), gently tap the plate frame for a few seconds to ensure thorough mixing.
7. Measure absorbance of each well at 450 nm within 30 minutes.

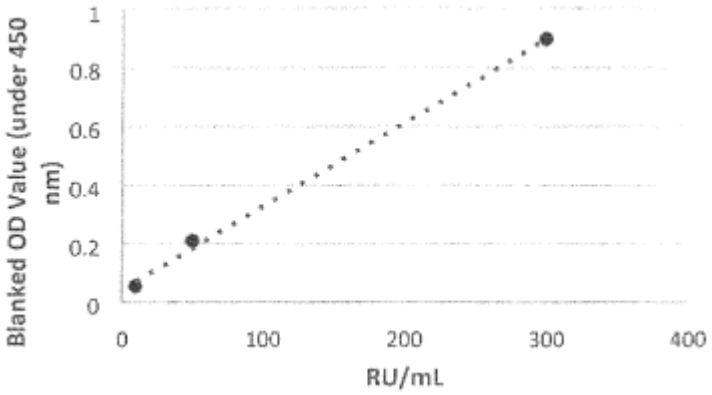
DATA INTERPRETATION

- Semi-quantitative:

Comparing the OD value of the sample with the calibrators:
  - Calibrator l - no specific suggestion
  - Calibrator 2 - suggestion for seeking a doctor
  - Calibrator 3 - suggestion for treatment

- Quantitative:
  1. Subtract the absorbance of the blank wells from that of standards and samples.
  2. Generate a standard curve by plotting the absorbance obtained (OD value, y-axis) against the concentration of the 3 Calibrators (RU/mL, x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
  3. Determine anti-histone antibody concentrations of samples from standard curve.
  4. The cutoff value is set to 42 RU/mL.

STANDARD CURVE



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ASSAY CHARACTERISTICS

A. Linearity

The linearity of Anti-dsDNA ELISA (IgG) was determined by assaying 8 serial dilutions of5 serum samples. The linear regression was calculated, R2 amounting to >0.98 within the concentration range of 10 RU/mL to 300 RU/mL.

B. Reproducibility

The reproducibility of the test was investigated by determine the intra- and inter-assay coefficients of variation (CV) using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different plates.

	Intra-assay Variation, n=20		Inter-assay variation, n=4x6	
Serum	**Mean Value (IU/mL)**	**CV (%)**	**Mean Value (IU/mL)**	**CV (%)**
1	82.57 ± 3.39	4%	63.77 ± 5.72	9%
2	222.41 ± 9.98	3%	173.15 ± 13.94	8%
3	418.77 ± 13.23	5%	362.63 ± 27.29	8%

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