Human Anti-Histones (IgG) ELISA Assay Kit

Catalog Number: HST31-K01 (1x 96 Wells)

For Research Use Only

v. 1.0 (effective 07 SEP 2023)

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INTENDED USE

The Eagle Biosciences Human Anti-Histone (IgG) ELISA Assay Kit 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. This kit is for research use only and not for use in diagnostic procedures.

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 BACKGROUND

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.

ASSAY PRINCIPLES

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 goat anti-human IgG horseradish peroxidase (HRP) conjugates are added, which bind to the captured anti histone 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 Anti-Nuclear 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.

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 anti histone antibodies, 12 strips (8 wells/strip), sealed
2. 5×Sample 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, ready for use
7. Negative control, human sourced, ready for use
8. 10×Wash buffer, 50 mL
9. Goat anti-human IgG-HRP solution, 12 µL, ready for use
10. Substrate solution, 12 mL, ready for use
11. Stopping solution, 2 M H_2SO_4, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. 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

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.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. Sample preparation

Vortex and centrifuge the sample tubes with a microcentrifuge at 16,000×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.

B. 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.

C. 1×Wash buffer

Prepare 1×Wash buffer by diluting the 10×Wash buffer (50 mL) with 450 mL of distilled/deionized water (v/v = 1:9). If crystals are observed in the 10×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×Wash buffer can be stored at 2-8°C for up to one month.

D. Goat anti-human IgG - HRP solution

Vortex the bottle to ensure the liquid is fully mixed before use. Dilute the anti-human IgG-HRP in 1×Sample buffer. Once the bottle is open, store at 2-8°C. E. 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.

F. Stopping solution

Stopping solution contains 2 M H_2SO_4, 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. 2. Discard the content and tap the plate on a clean paper towel to remove residual liquid in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×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 Goat 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 100 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 1 – no specific suggestion

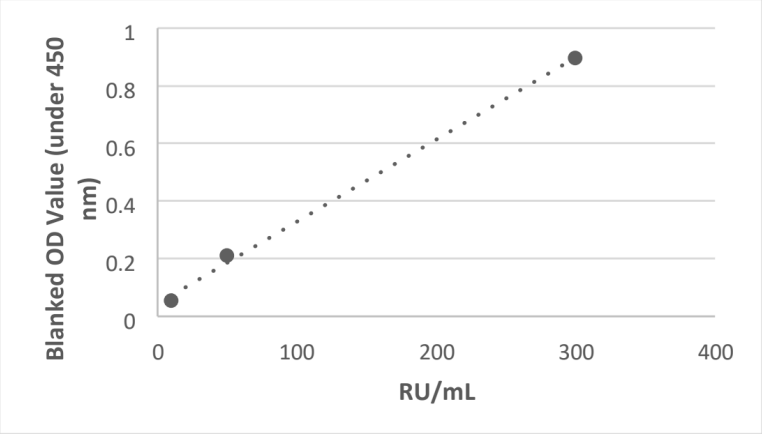
> Calibrator 2 – suggestion for seeking a doctor

> 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.

TYPICAL STANDARD CURVE



ASSAY CHARACTERISTICS

A. Linearity

The linearity of Anti-dsDNA ELISA (IgG) was determined by assaying 8 serial dilutions of 5 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=4 x 6**	
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%

REFERENCES

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2. DOOLEY, M. A. (2016). DRUG-INDUCED LUPUS. IN SYSTEMIC LUPUS ERYTHEMATOSUS (PP. 473-479). ACADEMIC PRESS.
3. FIRESTEIN, G. S., BUDD, R., GABRIEL, S. E., MCINNES, I. B., & O'DELL, J. R. (2016). KELLEY AND FIRESTEIN'S TEXTBOOK OF RHEUMATOLOGY E-BOOK. ELSEVIER HEALTH SCIENCES.
4. PORTANOVA, J. P., ARNDT, R. E., TAN, E. M., & KOTZIN, B. L. (1987). ANTI HISTONE ANTIBODIES IN IDIOPATHIC AND DRUG-INDUCED LUPUS RECOGNIZE DISTINCT INTRAHISTONE REGIONS. THE JOURNAL OF

IMMUNOLOGY, 138(2), 446-451.
5. PORTANOVA, J. P., RUBIN, R. L., JOSLIN, F. G., AGNELLO, V. D., & TAN, E. M. (1982). REACTIVITY OF ANTI-HISTONE ANTIBODIES INDUCED BY PROCAINAMIDE AND HYDRALAZINE. CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY, 25(1), 67-79.

PRECAUTIONS

- This kit is for research use only
- Compare contents and packing list, if there is breakage or shortage, notify Eagle Biosciences immediately
- Do not pipette reagents by mouth
- Do not smoke, eat or drink while performing assay
- Wear disposable gloves and proper lab protection and attire
- Treat all samples as potentially infectious
- Do not mix reagents from other lots
- Avoid contact with TMB and Stop solutions. If contact occurs, rinse thoroughly with water
- Eagle Biosciences is not responsible for outcomes as results of tampering with the reagents or using them unconventionally

SUMMARY OF ASSAY PROCEDURE

Dilute the sample with Sample buffer (1:100).

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Add 100 µL of calibrators, controls or samples to each well.

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Incubate at room temperature for 30 minutes.

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Discard all the liquid.

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Wash the wells with 300 µL of 1×Wash buffer for 3 repeats.

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Add 100 µL of Goat anti-human IgG-HRP solution to each well.

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Incubate at room temperature for 1 hour.

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Discard all the liquid.

↓

Wash the wells with 300 µL of 1×Wash buffer for 3 repeats.

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Add 100 µL of Substrate solution to each well.

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Incubate at room temperature for 10 minutes.

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Add 100 µL of Stop solution to each well.

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Measure absorbance of each well at 450 nm.

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Data interpretation

WARRANTY INFORMATION

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