

## ELISA kits available from ADI (see details at the web site)

3100	Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3105	Human anti-dsDNA IgM ELISA Kit, 96 tests, Quantitative
3110	Human anti-dsDNA IgA ELISA Kit, 96 tests, Quantitative
3115	Human anti-ssDNA IgG ELISA Kit, 96 tests, Quantitative
3205	Human Anti-Nuclear Antibodies (ANA) ELISA Kit, 96 tests, Semi-
3210-SSA	Human anti-SS-A/Ro IgG ELISA Kit, 96 tests, Quantitative
3220-SSB	Human anti-SS-B/La IgG ELISA Kit, 96 tests, Quantitative
3250	Human Anti-thyroid peroxidase ELISA kit, Semi-Quantitative
3300	Human Anti-helicobacter pylori IgG ELISA kit, Semi-Quantitative
3300-100-SMG	Human Anti-Smith antigen (Sm) IgG ELISA kit, 96 tests, Quantitative
3300-110-SRG	Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit, 96 tests,
3300-120-RNG	Human Anti-RNP (RNP-70) IgG ELISA kit, 96 tests, Quantitative
3300-130-HNG	Human Anti-histones IgG ELISA kit, 96 tests, Quantitative
3300-140-SCG	Human Anti-Scl-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-150-JOG	Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-160-AFG	Human Anti-Alpha Fodrin IgG ELISA kit, 96 tests, Quantitative
3300-170-CLG	Human Anti-Cardiolipin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM	Human Anti-Cardiolipin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA	Human Anti-Cardiolipin IgA ELISA kit, 96 tests, Quantitative
3300-190-B2G	Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-195-B2M	Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative
3300-200-APS	Human Anti-Phospholipid Screen IgG/IgM ELISA kit, 96 tests,
3300-200-B2A	Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-210-PSS	Human Anti-Phosphatidyl serine IgG/IgM ELISA kit, 96 tests,
3300-215-PIS	Human Anti-Phosphatidyl Inositol IgG/IgM ELISA kit, 96 tests,
3300-220-PAS	Human Anti-Phosphatidic Acid IgG/IgM ELISA kit, 96 tests,
3300-230-APG	Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests, Quantitative
3300-235-APA	Human Anti-Prothrombin IgA ELISA kit, 96 tests, Quantitative
3300-240-AVA	Human Anti-Annexin V IgG ELISA kit, 96 tests, Quantitative
3300-250-ANG	Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit, 96
3300-255-PRG	Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG	Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative
3300-270-GBG	Human Anti-glomerular basement membrane (GBM) IgG ELISA kit, 96
3300-280-BPG	Human Anti-bactericidal permeability increasing (BPI) protein IgG
3300-290-ELG	Human Anti-Elastase IgG ELISA kit, 96 tests, Quantitative
3300-300-GLG	Human Anti-Gliadin IgG ELISA kit, 96 tests, Quantitative
3300-305-GLM	Human Anti-Gliadin IgM ELISA kit, 96 tests, Quantitative
3300-310-GLA	Human Anti-Gliadin IgA ELISA kit, 96 tests, Quantitative
3300-315-PRG	Human Anti-Parietal cell (alpha and beta subunits of the Parietal Cell
(H//K/ATPase) IgG	ELISA kit, 96 tests,
3300-320-ASC	Human Anti-ASCA (mannan from Saccharomyces cerevisiae) IgA/IgG
3300-330-ASG	Human Anti-Sperm IgG ELISA kit, 96 tests, Quantitative
3300-340-CCG	Human Anti-Cyclic Citrullinated Peptide (CCP) IgG ELISA kit, 96 tests,
3300-350-TPG	Human Anti-thyroid peroxidase (TPO) IgG ELISA kit, 96 tests,
3300-360-TGG	Human Anti-thyroglobulin (TG) IgG ELISA kit, 96 tests, Quantitative
3310	Human Anti-helicobacter pylori IgM ELISA kit, Semi-Quantitative
3320	Human Anti-helicobacter pylori IgA ELISA kit, Semi-Quantitative
3600-HIG	Human Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3610-MKG	Monkey Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3700-MIG	Mouse Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3710-MIM	Mouse Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
3750-RIG	Rat Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3760-RIM	Rat Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
4000	Mouse Anti-Myelin Oligodendrocyte protein (MOG35-55) Ig's ELISA kit,

Instruction Manual No. M-3300-130-HNG

## Human Anti-histones IgG ELISA kit, 96 tests, Quantitative

ELISA KIT Cat. No. 3300-130-HNG



For Quantitative Determination of Anti-histones IgG  
In Human Serum or plasma

*For In Vitro Research Use Only*



6203 Woodlake Center Drive • San Antonio • Texas 78244 • USA.  
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Components	Cat. No.
Purified histones (H1, h2a, H2b, H3, and H4) antigen coated microwell strips (96 wells)	3300131
Anti-histones IgG <b>Stds. A-F</b> in a buffer (0, 12.5, 25, 50, 100, and 200 U/ml) 6 vials x 1.5 ml each	3300132A-F
Anti- histones IgG <b>Negative Control</b> (1.5 ml each)	3300133N
Anti- histones IgG <b>Positive Control</b> (1.5 ml each)	3300134P
<b>Sample Diluent (5X)</b> , 20 ml (yellow color)	3300130-SD
<b>Wash buffer (50X)</b> , 20 ml	3300130-WB
Anti-hIgG <b>HRP Conjugate</b> , 15 ml (light red)	3300130-EC
<b>HRP Substrate Solution (TMB)</b> , 15 ml	3300130-TM
<b>Stop Solution</b> , 15 ml	3300130-SS
Complete Instruction Manual	M-3300-130-HNG

Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes. They are the chief protein components of chromatin, acting as spools around which DNA winds, and play a role in gene regulation. Without histones, the unwound DNA in chromosomes would be very long (a length to width ratio of more than 10 million to 1 in human DNA). Histones are found in the nuclei of eukaryotic cells, and in certain Archaea, namely Euryarchaea, but not in bacteria. The unicellular algae known as dinoflagellates are the only eukaryotes that are known to completely lack histones. Five major families of histones exist: H1/H5, H2A, H2B, H3, and H4. Their molecular weights range from 11 to 21 kDa. They contain a lot of basic amino acid residues which presumably interact with the negative charged groups of DNA. Furthermore they contain polar amino acid residues which may be important for their interaction among each other. Histones H2A, H2B, H3 and H4 are known as the core histones, while histones H1 and H5 are known as the linker histones. Two of each of the core histones assemble to form one octameric nucleosome core particle, and 147 base pairs of DNA wrap around this core particle 1.65 times in a left-handed super-helical turn. The linker histone H1 binds the nucleosome at the entry and exit sites of the DNA, thus locking the DNA into place[7] and allowing the formation of higher order structure.

All of the nucleic acids (DNA) of eukaryotic cells are associated with proteins. The complex structure of DNA and its associated small basic proteins, which are called histones, is known as chromatin. Both, the histones comprise about 50 percent of the total mass of eukaryotic chromosomes. The complex of DNA and the histones can be dissociated by treatment of the eukaryotic chromatin with salt or diluted acids. Five different types of histones are known. They are called H1, H2A, H2B, H3 and H4. In correlation to their fundamental function in the organisation of chromatin, the structure of all histones in all eukaryotes is highly conserved.. Antibodies to histones usually produce a homogeneous, rim or speckled pattern of nuclear staining in indirect immunofluorescence

#### NAME AND INTENDED USE

Anti-Histone is an indirect solid phase enzyme immunoassay (ELISA) for the measurement of IgG class autoantibodies to histone in human serum or plasma. The assay is intended for research use only.

#### Intra-Assay

Intra-Assay		
Sample	Mean (u/ml0	CV (%)
1	23	4.1
2	52	3.8
3	120	4.6

Inter-Assay		
Sample	Mean (u/ml0	CV (%)
1	25	5.3
2	54	4.6
3	124	4.9

#### Sensitivity

The lower detection limit for Anti-histones IgG has been determined at 1.0 U/ml.

#### Specificity

The microplate is coated with histones highly purified by affinity chromatography. The Anti- histones test kit is specific only for autoantibodies directed to histones. No cross reactivities to the other ENA or DNA antigens have been observed.

#### Calibration

Since no international reference preparation for anti-histone autoantibodies is available, the assay system is calibrated against the WHO reference preparation for human anti nuclear factor (homogenous), MRC 66/233. With the Anti-Histone kit this preparation is determined at a concentration of 100 U/ml.

#### LIMITATIONS OF PROCEDURE

The Anti-histones IgG ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

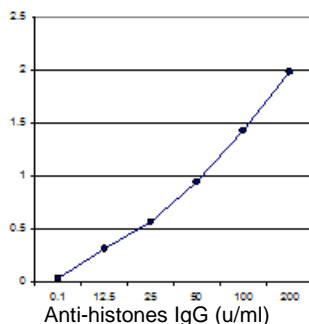
#### INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolysed or lipemic samples should be avoided.

**References:** Forelich H (1990) J. Rheumatol. 17, 192-200; Slobbe RI (1991) Clin. Exp. Immunol. 86, 99-105;

Typical Values of the standards (do not use this for calculations). These values are also lot specific and the following is only for demonstration purpose.

	Stds/samples	Mean OD450	Calculated Conc u/ml
A1/A2	0.00 u/ml	0.02	
B1/B2	12 u/ml	0.17	
C1/C2	25 u/ml	0.36	
D1/D2	50 u/ml	0.65	
E1/E2	100 u/ml	1.17	
F1/F2	200 u/ml	1.79	
S1/S2		0.63	49.58



#### Automation

The Anti-histones IgG ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

#### Quality Control

This test is only valid if the optical density at 450 nm for positive control (1) and negative control (2) as well as for the calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

## PRINCIPLE OF THE TEST

Anti-histones IgG ELISA kit is based on binding of anti- histones from serum samples to highly purified histones antigen immobilized on microtiter wells. After a washing step, goat anti-human IgG-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of anti- histones IgG present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of anti-histones IgG in samples is calculated using the reference standard curve.

## MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

## PRECAUTIONS

The ADI's ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Endpoint Cutoff and Positive controls have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

## SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

### Preparation of the reagent:

**Dilute wash buffer (1:50)** with distilled water (20 ml stock in total of 1-liter). store at 4oC.

**Sample buffer (1:5)** with distilled water (20 ml stock in total of 100-ml). store at 4oC for 30-day or until the expiration date printed on the label.

## REAGENT PREPARATION FOR THE ASSAY

1. Dilute wash buffer 1:50 (20 ml stock in 980 ml water) and store at 4°C.
2. Dilute sample diluent 1:5 (20 ml stock in 80-ml water) and store at 4°C.
3. Dilute all samples to be tested 1:100 with sample diluent (10 µl sample in 990 µl of diluent or 5 µl sample in 495 µl diluent).
4. Bring all reagents and samples to room temperature (25-30°C)

## STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions.

Do not contaminate the bottles. Withdraw solutions in a separate clean tube or dispensing trays. Any unused solution should be discarded and not returned to the bottle. Do not use HRP substrate solution if this solution is blue. Do not expose these solutions to strong light.

**TEST PROCEDURE** (*ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE*). .

1. Label, and secure the microtiter well strips to be used on the plate. **Dilute** samples (1:100) in 1x sample buffer. Controls provided in the kit are already pre-diluted. Dilute wash buffer (1:50) with distilled water (50 ml stock in total of 1-liter).
2. Pipet **100 µl of sample** buffer (for use as blanks), *pre-diluted* negative, positive controls, and *diluted* serum samples into appropriate wells in *duplicate*. Mix gently for 5-10 seconds, cover the plate and incubate for **30 minutes** at room temp (24-28°C).
3. Aspirate and **wash the wells 3 times** with 300 µl of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **100 µl of enzyme conjugate** into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temp.
5. Aspirate and wash the wells **3 times** as above.
6. Dispense **100 µl TMB substrate per well**. Mix gently for 5 seconds. Cover the plate and incubate at room temp in the dark. for **15 minutes**. **Blue color** develops in positive wells.
7. Stop the reaction by adding **100 µl** of stopping solution to all wells at the same timed intervals. Mix gently for 5-10 seconds. **Blue color turns yellow**. Measure the absorbance of yellow color at 450 nm using an ELISA reader.

**NOTES:** Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence.

## QUALITY CONTROL

This test is only valid if the optical density at 450 nm for positive control (1) and negative control (2) as well as for the calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

### Calculation of results

For Anti-histones a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Multiply the sample values only if the samples were diluted more than 1:100 (e.g., if sample diluted by 1:500 then multiply the values by 5).

### Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.