Histone Ab ELISA Kit

Catalog Number KA1120

96 assays

Version: 02

Intended for research use only

Table of Contents

Introduction

Intended Use

Enzyme Immunoassay for quantitative determination of IgG Autoantibodies to Histone in human serum or plasma

Background

All of the nucleic acids (DNA) of eukaryotic cells is 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. Their molecular weights range from 11 to 21 kDa. In correlation to their fundamental function in the organisation of chromatin, the structure of all histones in all eukaryotes is highly conserved. 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.Antibodies to histones usually produce a homogeneous, rim or speckled pattern of nuclear staining in indirect immunofluorescence. Antibodies against the histones dimers H2A-H2B are observed within 20 to 50 percent in spontaneous systemic Lupus erythematosus and in 50 to 90 percent in Procainamide induced SLE. Compared to other autoantibodies anti-histone antibodies are relatively rare in spontaneous SLE. Autoantibodies to histones are not specific for SLE but are found also in drug induced LE and rheumatoid arthritis (RA). In drug induced LE histone antibodies are found in a three times higher incidence than in SLE.Most of the anti-histone antibodies in drug induced LE are of temporary character. They mainly disappear within a few months after treatment with the inducing drug. Determination of anti-histone antibodies is indicated in:

- ✓ drug induced Lupus (mainly after treatment with Procainamide, Isoniazide or Hydralazine)
- ✓ drug induced ANA without clinical SLE symptoms
- ✓ spontaneous systemic Lupus erythematosus
- ✓ ANA positive and seropositive rheumatoid arthritis
- ✓ ANA positive sclerodermia

Disease	dsDNA	ssDNA	Histone	SS-A	SS-B	Sm	RNP/Sm	Scl-79	Jo-1
Systemic lupus erythrematosus (SLE)	>90	>90	30-50	10-30	30-50	10-30	10-30		
Drug-induced lupus (DIL)		30-50	50-90						
Sharp-syndrome/mixed connective tissue disease	10-30	10-30					>90		
Rheumatoid arthritis	10-30	30-50	30-50	10-30					
Sjogren's syndrome	10-30	10-30		>90	>90				
Scleroderma	10-30	10-30		10-30				>90	
Photosensitive dermatitis, dermatomyositis	10-30	10-30							50-90

Principle of the Assay

Highly purified total histones are bound to microwells. Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

General Information

Materials Supplied

List of component

Component	Amount
Divisible microplate: Consisting of 12 modules of 8 wells each. Ready to use.	96 (8x12) wells
Calibrators A-F (0, 12.5, 25, 50, 100, 200 U/ml), containing serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%),yellow. Ready to use.	1.5 ml x 6
Control positive and (1) negative (2), containing histone antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%, yellow. Ready to use.	1.5 ml x 2
Sample buffer P, containing PBS, BSA, detergent, preservative NaN_3 0.09%, yellow, 5x conc.	20 ml
Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.	15 ml
TMB Substrate; containing 3,3',5,5'-Tetramethylbenzidin, colorless. Ready to use.	15 ml
Stop solution (contains acid). Ready to use.	15 ml
Wash solution, containing Tris, detergent, preservative NaN_3 0.09%; 50 x conc.	20 ml

Storage Instruction

- ✓ Store the kit at 2-8°C.
- ✓ Do not expose reagents to heat, sun or strong light during storage and usage.
- ✓ Store microplate sealed and desiccated in the clip bag provided.
- ✓ Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- ✓ Diluted Wash Solution and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

Materials Required but Not Supplied

- ✓ Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm.
- ✓ Data reduction software
- ✓ Multi-Channel Dispenser or repeatable pipet for 100 µl
- ✓ Vortex mixer
- ✓ Pipets for 10 µl, 100 µl and 1000 µl
- ✓ Laboratory timing device
- ✓ Distilled or deionized water
- ✓ Measuring cylinder for 1000 and 100 ml
- ✓ Plastic container for storage of the wash solution

Precautions for Use

✓ Warning and Precautions

- All reagents of this kit are intended for research use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop Solution contains acid, classification is non-hazardous. Avoid contact with skin.
- Controls, calibrator, sample buffer and wash solution contain sodium azide (NaN_3) 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
- During handling of all reagents, controls and serum samples observe the existing regulations for laboratory sfety regulations and good laboratory practice:
  First aid measures: In case of skin contact, immediately wash thoroughly with water and soap.
  Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:
  Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- Exposure controls/personal protection: Wear protective gloves of nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.
- Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

✓ Warning and Precautions

- This EILSA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system.
- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.

- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash solution.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

Assay Protocol

Reagent Preparation

✓ Wash Solution

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionisedwater toa final olume of 1000 ml prior to use.

✓ Sample buffer P

Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionized water to a final volume of 100 ml.

Sample Preparation

✓ Specimen Collection, Storage and Handling

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

✓ Preparation of Samples

Dilute samples 1:100 with sample buffer before assay. Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. *Note: Calibrators/Controls are ready to use and need not be diluted.*

Assay Procedure

1. Prepare enough microplate modules for all calibrators/controls and samples.
2. Pipette 100 µl of calibrators, controls and prediluted samples into the wells.
3. Incubate for 30 minutes at room temperature (20-28°C).
4. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
5. Dispense 100 µl of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
8. Dispense 100 µl of TMB substrate solution into each well.

9. Incubate for 15 minutes at room temperature.
10. Add 100 µl of stop solution to each well of the modules
11. incubate for 5 minutes at room temperature.
12. Read the optical density at 450 nm (reference 600-690 nm) and calculate the results. The developed color is stable for at least 30 minutes. Read optical densities during this time.

Data Analysis

Calculation of Results

- ✓ Calculation of results

  For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of samples may then be estimated from the calibration curve by interpolation.

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

Performance Characteristics

- ✓ Calibration

  This assay system is calibrated against the international reference preparation WHO MRC 66/233 for human anti-nuclear factor (homogenous) as 100 U/ml..

- ✓ Measuring range

  the calculation range of this ELISA assay is 0-200 U/ml.

- ✓ Expected values

  In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 10 U/ml.

- ✓ Interpretation of results

  Negatie: < 40 U/ml

  Positive: ≥ 40 U/ml

- ✓ Limit of detection

  Functional sensitivity was determined to be: 1 U/ml.

- ✓ Interfering substances

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

✓ Linearity

Samples containing high leels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper/lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	169.7	169.7	100
	1:200	83.7	84.9	99
	1:400	44.0	42.4	104
	1:800	22.1	21.2	104
2	1:100	190.0	190.0	100
	1:200	92.1	95.0	97
	1:400	46.5	47.5	98
	1:800	23.2	23.8	98

✓ Reproducibility

Intra-assay precision: Coefficient of ariation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean U/ml	CV%
1	223.0	4.1
2	52.0	3.8
3	120.0	4.6

Inter-Assay		
Sample	Mean U/ml	CV%
1	25.0	5.3
2	54.0	4.6
3	124.0	4.9

Resources

References

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Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard A	Sample 1										
B	Standard B	Sample 2										
C	Standard C	Sample 3										
D	Standard D											
E	Standard E											
F	Standard F											
G	Control +											
H	Control -											