Nucleosomes Antibody ELISA Kit

Cat. No.: DEIA1831
Pkg. Size: 96T

**Intended use**

The kit is an indirect solid phase enzyme immunoassay (ELISA) for measurement of IgG class autoantibodies against Nucleosomes in human serum or plasma.

**General Description**

A nucleosome is the basic unit of DNA packaging in eukaryotes, consisting of a segment of DNA wound in sequence around eight histone protein cores. This structure is often compared to thread wrapped around a spool. Nucleosomes form the fundamental repeating units of eukaryotic chromatin, which is used to pack the large eukaryotic genomes into the nucleus while still ensuring appropriate access to it (in mammalian cells approximately 2 m of linear DNA have to be packed into a nucleus of roughly 10 µm diameter). Nucleosomes are folded through a series of successively higher order structures to eventually form a chromosome; this both compacts DNA and creates an added layer of regulatory control, which ensures correct gene expression. Nucleosomes are thought to carry epigenetically inherited information in the form of covalent modifications of their core histones. The nucleosome hypothesis was proposed by Don and Ada Olins in 1974 and Roger Kornberg.

**Principle Of The Test**

Human Nucleosomes are bound to microwells. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated antihuman IgG immunologically detects the bound specimen sample 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 color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.

**Reagents And Materials Provided**

1. Divisible microplate consisting of 12 modules of 8 wells each, coated with human Nucleosomes. Ready to use; 1 plate
2. Combined Calibrators with IgG class Anti-Nucleosome antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaCN<0.1% (w/w)). Ready to use; 6 vials, 1.5 ml each containing: IgG: 0; 12.5; 25; 50; 100; and 200 U/ml.
3. Anti-Nucleosome Controls in a serum/buffer matrix (PBS, BSA, NaCN<0.1% (w/w)) positive and negative, for the respective concentrations see the enclosed QC insert. Ready to use; 2 vials, 1.5 ml each
4. Sample buffer (Tris, NaCN<0.1% (w/w)), yellow, concentrate (5x); 1 vial, 20 ml
5. Enzyme conjugate solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG; labelled with horseradish peroxidase. Ready to use; 1 vial, 15 ml
6. TMB substrate solution. Ready to use; 1 vial, 15 ml
7. Stop solution (contains acid). Ready to use; 1 vial, 15 ml
1 vial, 20 ml Wash solution (PBS, NaCN<0.1% (w/w)), concentrate (50x); 1 vial, 20 ml

**Materials Required But Not Supplied**
1. Microplate reader capable of endpoint measurements at 450 nm
2. Multi-Channel Dispenser or repeatable pipette for 100 µl
3. Vortex mixer
4. Pipettes for 10 µl, 100 µl and 1000 µl
5. Laboratory timing device
6. Data reduction software
7. Distilled or deionised water
8. Graduated cylinder for 100 and 1000 ml
9. Plastic container for storage of the wash solution

Storage

1. Store the kit at 2°C - 8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2°C - 8 °C.

Specimen Collection 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.

Reagent Preparation

Sample Buffer
Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use.
Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Wash Solution
Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use.
Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample Preparation
Dilute all specimen sample samples 1:100 with sample buffer before assay.
Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.
Controls are ready to use and need not be diluted.

Assay Steps

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted specimen sample samples.
2. Pipet 100 µl of calibrators, controls and prediluted specimen sample samples in duplicate 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 and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

**Calculation**

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

**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.

**Typical Standard Curve**

The figures below show typical results for Anti-Nucleosome ELISA. These data are intended for illustration only and should not be used to calculate results from another run.

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<thead>
<tr>
<th>Calibrators</th>
<th>No</th>
<th>Position</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean</th>
<th>Conc. 1</th>
<th>Conc. 2</th>
<th>Mean</th>
<th>decl. Conc.</th>
<th>CV %</th>
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<td>0.0</td>
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<tr>
<td>ST2 B 1/B 2</td>
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<td>0.174</td>
<td>13</td>
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<tr>
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**Precautions**

1. Do not interchange kit components from different lots.
2. 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 and HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
3. Avoid contact with the TMB (3,3’,5,5’-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
4. Avoid contact with the Stop Solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
5. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN₃) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).
6. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
7. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
8. Do not pipette by mouth.
9. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
10. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

REFERENCES