

NUCLEOSOME ANTIGEN

AROTEC_Nucleosome_Product_Info.pdf Version/Date: A/00.08.18

ATN02-02	Nucleosome antigen	0.20 mg
ATN02-05	Nucleosome antigen	0.50 mg
ATN02-10	Nucleosome antigen	1.0 mg

Description of the Product

Purified from bovine thymus. After coating onto ELISA plates the product will bind autoantibodies to nucleosome antigen.

Purity: The nucleosome antigen is more than 90% pure, as assessed by SDS gel electrophoresis.

Concentration: 0.5 -2.0 mg protein/ml.

Storage: The product is stabilised with 0.1% Micr-O-protect™. Store at -20 °C or below (long term) or at +4°C (short term). Avoid repeated freezing and thawing. Mix thoroughly before use.

Clinical and Biochemical Data

Anti-nucleosome antibodies (also referred to as anti-chromatin antibodies) constitute one of the first autoantibody specificities found to be present in systemic lupus erythematosus (SLE) having been detected as early as 1948 using the LE cell test^{1,2}. The prevalence of anti-nucleosome antibodies in SLE is of the order of 50-100%², and the nucleosome has been identified as the initiating and driving immunogen in SLE^{3,4}. Antinucleosome antibodies are now considered to be a more sensitive marker for SLE than anti-dsDNA antibodies⁵ and a high anti-nucleosome antibody titre has been reported to be indicative of lupus nephritis^{6,8}. Anti-nucleosome antibodies have been found (with a lower prevalence than in SLE) in a number of other autoimmune diseases^{6,9,10,19} such as systemic sclerosis, Sjogren's syndrome and mixed connective tissue disease and are also found in 40-50% of patients with autoimmune hepatitis type 1^{11,12}. Anti-ribosomal P antibodies have also been reported to bind to nucleosomes^{13,14}.

The nucleosome is the basic structural subunit of chromatin, the native complex of histones and DNA found in the nucleus of eukaryotic cells. It is composed of about 200 base pairs of DNA wrapped twice around a (H2A-H2B-H3-H4)₂ histone octamer with histone H1 bound on the periphery^{2,15,16,19}. Nucleosomes can be isolated by digesting the linker DNA holding them together in chromatin with micrococcal nuclease. The most effective form of nucleosomes for detecting patient autoantibodies are prepared in this way and stripped of H1 histone to yield a nucleosome core particle in which 146 base pairs of DNA are wrapped around an octamer of two H2A-H2B dimers that surround an H3-H4 tetramer^{2,17}. Autoantibodies against subnucleosome particles occur in SLE, they are however less frequent³.

AROTEC nucleosome antigen is prepared from calf thymus chromatin and contains the four core histones H2A, H2B, H3 and H4 bound to DNA of about 146 base pairs in length. Histone amino acid sequences are highly conserved between species, even between animals and plants¹⁸. Homology between human and bovine amino acid sequences is as follows (ExPASy accession numbers human:bovine are shown):

H2A (P0C0S5:P0C0S4)	128 aa: 128 identical
H2B (B2R4S9:A5D7N2)	126 aa: 125 identical, 1 conservative substitution
H3 (P84243:Q5E9F8)	136 aa: 136 identical
H4 (P62805:P62803)	103 aa: 103 identical

Methodology

The following is an ELISA procedure which can be used to detect anti-nucleosome autoantibodies in human serum using the ATN02 purified nucleosome antigen:

1. Dilute the purified antigen to 1.0-2.0 µg/ml in 20 mM Tris/HCl buffer, pH 8.0; containing 0.15 M NaCl.
2. Coat ELISA plates with 100 µl of diluted antigen per well. Cover and incubate 24 hours at +4°C.
3. Empty the plates and remove excess liquid by tapping on a paper towel.
4. Block excess protein binding sites by adding 200 µl PBS containing 1% BSA per well. Cover and incubate at +4°C overnight.
5. Empty plates and apply 100 µl of serum samples diluted 1:100 in PBS / 1% BSA / 0.1% Tween® 20. Incubate at room temperature for 1 hour.
6. Empty plates and add 200 µl PBS / 0.1% Tween® 20 per well. Incubate 5 minutes then empty plates. Repeat this step twice.
7. Apply 100 µl anti-human IgG-enzyme conjugate (horseradish peroxidase or alkaline phosphatase) diluted in PBS / 1% BSA / 0.1% Tween® 20 per well and incubate for 1 hour.
8. Repeat step 6.
9. Add enzyme substrate and stop the reaction when appropriate.
10. Read absorbance in an ELISA spectrophotometer.

References

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NOTE: **No patented technology** has been used by AROTEC during the preparation of this product.



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