

HISTONE ANTIGEN

AROTEC_Histone_Product_Info.pdf Version/Date: A/00.06.02

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| ATH01-02 | Histone antigen | 0.20 mg |
| ATH01-05 | Histone antigen | 0.50 mg |
| ATH01-10 | Histone antigen | 1.0 mg |

Description of the Product

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

Purity: The histone autoantigen 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

Autoantibodies against histones (AHAs) are observed in a number of autoimmune diseases. AHA are reported in 50-80% of patients with Systemic Lupus Erythematosus (SLE) being highest in patients with active disease¹. Although H1 and H2B are the most common epitopes in SLE, many SLE patients have conformation-dependent AHA, directed against the histone complex². AHA have particular clinical significance for drug-induced lupus, particularly in the diagnosis of antinuclear antibody positive patients receiving procainamide, hydralazine and isoniazide³. AHA are also prevalent in Felty's syndrome (83%), rheumatoid arthritis (75%) and juvenile arthritis (50-75%)², scleroderma^{4,5} systemic sclerosis⁶ and mixed connective tissue disease⁷. AHA (predominantly against H1) are also observed in approximately 76% of patients with primary biliary cirrhosis².

Histones are small DNA-binding proteins and the major protein component of the nucleosome. The nucleosome consists of 146 base pairs of DNA wrapped around an octamer of core histone proteins composed of a central tetramer of two H3-H4 dimers flanked by two H2A-H2B dimers⁸. Histone H1 is a linker histone, present between each nucleosome, and is responsible for establishing chromatin structure. The molecular weights of the core histones range from 11,000 to 15,000. Histone H1 is larger, with a molecular weight of 23,000. All of the histones contain many basic amino acids, with histones H3 and H4 being arginine rich, while H2A and H2B are slightly lysine-rich⁸.

Biochemical and serological studies have revealed a more complicated picture of histone antigenicity than initially recognised, particularly highlighted by observations whereby separation of the histone complex into its individual components using harsh conditions can result in a loss of complex formation and antibody reactivity^{9,10}. Histone epitopes are often located in accessible regions of chromatin (especially for H1 and H2A/H2B)¹¹ or are conformational determinants resulting from the association of several histone components. Acetylation of histone lysine residues may also play a role in SLE autoantigenicity¹². The H2A-H2B dimer is the main antigen in drug-induced lupus, although also observed in SLE, whereas linear epitopes are generally only observed for core histones².

Histone amino acid sequences are highly conserved between species, even between animals and plants¹³. AROTEC histone antigen is prepared from calf thymus chromatin and contains the five main histones, H1, H2A, H2B, H3 and H4, extracted and purified without the use of harsh denaturing reagents to ensure maximum reactivity with human autoantibodies.

Methodology

The following is an ELISA procedure which can be used to detect anti-histone autoantibodies in human serum using the ATH01 purified histone antigen:

1. Dilute the purified antigen to 1.0-2.0 µg/ml in 50 mM carbonate buffer, pH 9.6 containing 0.5% (w/v) sodium deoxycholate.
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.