Kreative Technolabs

DATA SHEET

MYELOPEROXIDASE (pANCA) ANTIGEN

Code Number#KT-ATM01-02

Pack Size: 0.20 mg

Description: Purified from human neutrophils from blood tested and found to be negative for HBs-antigen, anti-HIV1, anti-HIV2, anti-HCV, Lues and GPT. After coating onto ELISA plates the product will bind autoantibodies to myeloperoxidase (pANCA) antigen.

Purity: > 95%, as assessed by SDS gel electrophoresis.

Storage: Store at -65 ^{o}C or below (long term). Avoid repeated freezing and thawing.

General: Autoantibodies to neutrophil cytoplasmic antigens (ANCA) were first described in 1982 by Davies et al. Autoantibodies staining the nuclei or the perinuclear zone of neutrophils by indirect immunofluorescence are referred to as pANCA whereas those giving a clear cytoplasmic fluorescence are referred to as cANCA. The antigen recognised by most pANCA sera has been identified as myeloperoxidase. Autoantibodies to myeloperoxidase are found in the sera of patients with various types of systemic vasculitis including idiopathic crescentic glomerulonephritis, Churg-Strauss syndrome, microscopic polyangiitis and polarteritis nodosa. Anti-myeloperoxidase antibodies have also been reported in some patients with Wegener's granulomatosis, and occasionally in patients with rheumatoid arthritis or inflammatory bowel disease.

The use of purified myeloperoxidase for the detection of anti-myeloperoxidase autoantibodies by solid-phase ELISA has been described by several authors.

Application: **ELISA.**

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

1. Dilute the purified antigen to 0.5-1.0 µg/ml in 0.05 M carbonate buffer pH 9.5.
2. Coat ELISA plates with 100 µl of diluted antigen per well. Cover and incubate overnight at room temperature.
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 (10 mM potassium phosphate, pH 7.4, 0.15 M NaCl) containing 1% BSA per well. Incubate at room temperature for three hours.
5. Empty plates and apply 100 µl of serum samples diluted 1:100 in PBS / 1% BSA / 1% casein / 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 / 1% casein / 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.

NOTE: **For In-Vitro Diagnostic Use. Not For Human Or Animal Consumption**.

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