Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit, 96 tests, Quantitative# 3300-250-ANG

This is a qualitative enzyme immunoassay (EIA) intended to screen for the presence of IgG class autoantibodies against PR3 and MPO in human serum or plasma. For in vitro research use only.

ELISA Kit Features

- microplate consisting of 12 X 8 wells each, coated with a mixture of highly purified antigens, PR3 and MPO. Ready to use.
- Negative, positive, and Cut-off control; Sample Buffer, Enzyme conjugate, TMB, Stop solution and Wash solution.
- Sample size 100 ul: Serum or plasma; (diluted).
- 65 minutes, 4 incubation steps
- Contains all necessary reagents.
- Sample values are calculated from the standard curve.

Assay Procedure: Allow all reagents to reach room temperature. Arrange and label required number of strips.

Step 1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted samples.
Step 2. **Pipette 100 µl of controls and prediluted samples in duplicate into the wells** and incubate at **RT** for **30 mins.**
Step 3. **Decant and Wash 3X**. **Add 100 ul enzyme conjugate** to each well and **incubate at RT** for **15 mins.**
Step 4. **Decant and Wash 3X. Add 100 ul TMB Substrate Solution to each well and incubate at RT for 15 mins.**
Step 5. Pipet **100 ul of stop solution** into each well and mix gently (blue color turns yellow). Incubate at **RT for 5 mins**. **Measure absorbance at 450 nm**.

Performance characteristics

Evaluation of the ANCA screen test is easily carried out by direct comparison of the optical density of each unknown with the optical density of the Cut-Off Control. Unknowns exhibiting optical densities higher than the optical density of the Cut-Off Control are considered to be positive.

Negative: OD Sample < OD Cut-Off Control
Positive: OD Sample ≥ OD Cut-Off Control

For detailed quantification of the results, each OD value can be expressed by the "Index Value". The Index Value is calculated by dividing the sample-OD by the Cut-Off-OD.

$$\text{Index Value} = \frac{OD_{Sample}}{OD_{Cut\text{-}Off}}$$

The calculation of Index Values is not influenced by variations of the sample-OD and/or Cut-Off-OD. Index Values are recommended for long term validations (i.e. internal quality control samples). The calculation of Index Values is not influenced by variations of the sample-OD and/or Cut-Off-OD. Index Values are recommended for long term validations (i.e. internal quality control samples). Further differentiation and typing should be carried out by using the quantitative Anti-PR3 and Anti-MPO ELISA.

Precision:
Intra-assay (%CV) MPO: 6.3-9.1; PR**3**: 5.5-7.2**; Inter-assay (%CV) : MPO**: 5.5-7.2; **PR3**: 6.9-9.8

Specificity & Species reactivity: The microplate is coated with a mixture of PR3 and MPO antigens, highly purified by affinity chromatography. The ANCA screen test is specific only for autoantibodies directed to these antigens. No cross-reactivities have been observed.

Principle

A mixture of highly purified PR3 and MPO antigens is bound to microwells. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigens. After washing and addition of Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing and addition of 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 450nm.The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

Related Items

Catalog#	Product Description
3300-255-PRG	Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG	Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative

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