Human Anti-ASCA (mannan from Saccharomyces cerevisiae) IgA/IgG ELISA kit
3300-320-ASC

This kit is for the determination of Anti- Saccharomyces cerevisiae antibodies (ASCA) of the IgG and IgA class in human serum.
For in vitro research use only.

- **PRINCIPLE OF THE TEST**

Highly purified mannan from Saccharomyces cerevisiae is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigens. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG or IgA immunologically detect the bound 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 colour product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG resp. IgA antibodies present in the original sample.

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

Step 1. Pipet **100 ul each** of controls and prediluted patient samples in duplicate into the wells. Mix gently and **incubate at RT for 30 mins.**
Step 2. **Aspirate and Wash 3X** Add **100 ul** of **HRP** solution, cover and incubate at **RT** for **15 mins.**
Step 3. **Aspirate and Wash 3X**. **Add 100 ul TMB substrate solution** to all wells, mix gently, and **incubate at RT** for **15 mins .**
Step 4. 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**. Determine the conc. in each sample using the standards

Performance characteristics

Sensitivity
The lower detection limit for ASCA ELISA was determined at 1 U/ml.

Specificity: The solid phase is coated with mannan from Saccharomyces cerevisiae. Therefore the ASCA test kit recognizes only autoantibodies specific for this phosphopeptide.

Calibration: Since no international reference preparation for ASCA is available, the assay system is calibrated in relative arbitrary units.

INTERFERING SUBSTANCES: No interference has been observed with hemolytic, lipemic or bilirubin containing sera. Nor have any interfering effects been noticed with the use of anticoagulants.

CONTENTS OF THE KIT

divisible microplate consisting of 12 modules of 8 wells each, coated with highly purified mannan from Saccharomyces cerevisiae. Ready to use.

Calibrators with IgG and IgA class ASCA (A-F) in a serum/buffer matrix containing IgG: 0; 6.3; 12.5; 25; 50; 100 U/ml, IgA: 0; 6.3; 12.5; 25; 50; 100 U/ml. Ready to use.

ASCA controls in a serum/buffer matrix ; positive (1) and negative (2), for the respective concentrations see the enclosed package insert. Ready to use.

Sample buffer (5x).
Enzyme conjugate solution containing polyclonal rabbit anti-human IgG; labelled with horseradish peroxidase. Ready to use.
Enzyme conjugate solution containing polyclonal rabbit anti-human IgA; labelled with horseradish peroxidase. Ready to use.
TMB substrate solution. Ready to use.
Stop solution (contains acid). Ready to use.
Wash solution concentrate (50x).

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