Candida albicans IgG ELISA Kit
Quantitative assay for Candida albicans IgG

**REF GD022-RUO**

For Research Use Only. Not For Use in Diagnostic Procedures.

1. **Materials Included in the Kit**
   - [MTF] 96 wells in 12 X 8 break-apart strips, pre-coated with C. albicans antigens, with holder in a foil bag with desiccant
   - [DIL] 100mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 100mL (blue), ready to use
   - [WB] 100mM Tris-buffered saline with detergent, pH 7.2, 100mL, concentrate (x10)
   - [CONJ] rabbit anti-human IgG (red) conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12mL, ready to use
   - [SUBS] aqueous solution of TMB and hydrogen peroxide, 12mL, ready to use
   - [STOP] 0.25M sulphuric acid, 12mL, ready to use
   - [STD[1..5]] 0, 12.5, 25, 50 & 100U/mL, 1mL of 10mM Tris-buffered saline containing human serum IgG antibodies to C. albicans, ready to use
   - [PC] 1mL of 10mM Tris-buffered saline containing either human serum IgG antibodies to C. albicans, ready to use

2. **Other Equipment Required**

**GENESIS Diagnostics**

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Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 5µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable, self-validated automated system may be used.

Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

3. Intended Use
The Candida albicans IgG kit is a rapid ELISA methods for the detection of IgG antibodies to Candida albicans. It is intended for the identification of Candida albicans infection. The components of the kit are for research use only.

4. Explanation of the Test
A number of fungal pathogens can spread systemically from the intestinal lumen to the visceral organs. Although hundreds of Candida species are known, C. albicans and C. tropicalis cause over 80% of Candida infections in man. Candida probably enters the newborn in the first days of life and is a normal inhabitant of the gut. Systemic candidiasis is a fungal infection of the deep organs resulting from the overgrowth and spread of Candida. It is a significant cause of death in immuno-compromised patients or those undergoing prolonged antibiotic therapy. Candida infection is not routinely tested for in blood donors and may also be transmitted via blood transfusions. Elevated Candida IgG levels are also frequently encountered in elderly patients (>60 years).

Recent infection with systemic candidiasis is characterised by elevated IgM and IgG titres. Candida IgA antibodies are associated with mucosal membrane infections.

5. Principle of the Test
Diluted serum samples are incubated with C. albicans antigens immobilised on microtitre wells. After washing away unbound serum components, rabbit anti-human IgM conjugated to horseradish peroxidase is added to the wells, and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of stop solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the standards, positive control and samples are measured using a microplate reader at 450nm.

1. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
2. CAUTION: the device contains material of human and animal origin and should be handled as a potential transmitter of diseases. All human source material used in the preparation of standards and control of this product have been tested and found negative by ELISA for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
3. Reagents of this kit contain antimicrobial agents and the substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
4. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
5. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Dispose of plates and specimens as clinical waste. Any unused reagents should be flushed away with copious amounts of water. Disposal must be performed in accordance with local legislation.

7. Technical Precautions
1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of stop solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the standards, positive control and samples are measured using a microplate reader at 450nm.

6. Safety Precautions
On arrival, store the kit at 2-8°C. Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer has a shelf life of 3 months if stored in a closed bottle at 2-8°C.

9. Specimen Collection and Storage
Serum and plasma samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbiologically contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

10. Preparation of Reagents
Dilute the Wash Buffer [WB] 1:9 in distilled water to make sufficient buffer for the assay run e.g. add 50mL wash buffer concentrate to 450mL water.

11. Assay Procedure
1. Dilute patient samples 1:200 in sample diluent (e.g. 5µl serum plus 1mL diluent).
2. Allow the diluted samples to stand for 30 minutes. The sample diluent contains an immunosorbent that removes non-candida antibodies.
3. Assemble the number of strips required for the assay.
4. Dispense 100µL of each standard, and positive control and the diluted patient samples into appropriate wells.
5. Incubate for 30 minutes at room temperature.
6. After 30 minutes, decant or aspirate the well contents and wash the wells 3 times using automated washing or the manual wash procedure (see below). Careful washing is the key to good results. Do not allow the wells to dry out.

Manual Wash Procedure
Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.

7. Dispense 100µL of Conjugate [CONJ] into each well. Incubate the wells for 30 minutes at room temperature.
8. After 30 minutes, discard the well contents and carefully wash the wells 4 times with wash buffer. Ensure that the wells are empty but do not allow to dry out.
9. Using a repeating dispenser, rapidly dispense 100µL of TMB Substrate [SUBS] into each well. Incubate the plate for 10 minutes.

8. Shelf Life and Storage Conditions