Biocare™ Diagnostics

# Anti-TB IgG ELISA Kit

*Enzyme-linked immunosorbent assay for the detection of TB IgG in serum or plasma*

**INTRODUCTION**

The Mycobacterium tuberculosis IgG antibody ELISA kit has been designed for the detection and the quantitative determination of specific IgG antibodies against Mycobacterium tuberculosis in serum and plasma.

This assay is intended for in-vitro diagnostic use only.

Mycobacterioses (tuberculosis, leprosy, atypical mycobacterioses, paratuberculosis, and perhaps Crohn's Disease) are the infectious diseases of men and animals with the largest diffusion on earth. The infectious agents of tuberculosis are acid-resistant rod-like formed bacteria of the family Mycobacteriaceae, genus Mycobacterium. The germ was detected by Robert Koch in 1882. Owing to the very high infectious power of pathogenic mycobacteria, early diagnosis is essential to prevent spreading of the disease. Convergence of various approaches are necessary to control the mycobacterioses, immune reactions and bacterial shedding being variable during the diseases. However, usual diagnostic procedures were up to now unsatis-factory and did not allow to distinguish among different mycobacterial species.

The illness is normally transferred by droplets of saliva from infected persons. The target of the infection are mostly the lungs, but also other organs like the brain, intestinal tract, bones, lymph nodes and kidneys can be afflicted. Tuberculosis is not only found in developing countries with 8 million of new infections yearly, but also in industrialized civilizations, as an actual disease with some thousands of cases yearly. Without treatment, the disease leads in 50% of the cases to death within less than two years. Clinical symptoms are fatigue, loss of weight, lack of appetite, light fever, nocturnal sweat and pain in the chest. Especially patients with HIV are threatened by tuberculosis due to their impaired immune system.

A vaccination with living attenuated bacteria is possible (BCG = Bacille Calmette Guérin). This is mostly done with newborn or young children. With older patients, before the vaccination there is normally performed the tuberculin test (Pirquet or Mantoux), where a small amount of tuberculin is injected under the skin. In a positive case, there exist antibodies against Mycobacteria, and a vaccination is not necessary.

Up to recently, there have not existed any serological methods to detect tuberculosis antibodies in serum. The only available procedure was besides the skin tuberculin test the direct microscopical identification of the dyed bacteria in sputum. Meanwhile specific antigens have been prepared either by purification of natural material or by recombinant methods. This ELISA test kit for the determination of IgG antibodies uses a cocktail of highly pure proteins in order to determine an immune response against the bacteria in human serum.

**PRINCIPLE OF THE TEST**

Mycobacterium tuberculosis IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Mycobacterium tuberculosis antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Mycobacterium antigen takes place. After 30mins incubation, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 20 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 10 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

**REAGENTS**

***Materials provided with the kits:***

1. Microtiter Well: 8x12 or 12x8, coated with TB antigen
2. Negative Control: 0.5ml TB negative serum.
3. Positive Control: 0.5ml TB positive serum.
4. Enzyme Conjugate: 12 ml, Goat anti-human IgG-HRP
5. Sample Diluent: 12ml
6. Wash Buffer Concentrate (20x): 40 ml, The buffer should be diluted 20 times with distilled water before use.
7. Substrate Solution A: 6 ml Urea Peroxide
8. Substrate Solution B: 6 ml TMB solution
9. Stop Solution: 6 ml 2N Sulfuric Acid

***Materials required but not provided:***

1. Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader.

**SPECIMEN COLLECTION AND PREPARATION**

No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Plasma samples containing EDTA, heparin or oxalate may interfere with test procedures and

should be avoided. Specimen with extensive particulate should be clarified by centrifugation prior to use. Covered specimens may be stored for up to 48 hours at 2°-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C for mix prior to testing. Avoid repeated freeze thaw. At least, two wells of negative and positive controls each should be run in every assay.

**PRECAUTIONS**

1. Caution: Some components of this kit contain human serum. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
2. Wear disposable gloves while handling kit reagents and specimens and thoroughly wash hands afterwards.
3. Dispose off all specimens and materials used to perform the test as if they contained infectious agents.
4. Do not mix reagents from kits with different lot numbers.
5. Cross contamination between reagents will invalidate the test results.
6. All reagents and components except the conjugate must be equiliberated at room temperature prior to use.

**STORAGE OF TEST KITS AND INSTRUMENTATION**

Unopened test kits should be stored at 2°-8°C upon receipt. Micro titer plate, once opened, should be kept in a sealed bag with desiccants to minimize exposure to damp air. To remove the required number of strips from the micro titer plates, bring the sealed pouches to room temperature first and then open the pouches. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. Opened test kits will remain stable until the expiration date shown in 4°C, provided it is stored as described above. A micro titer plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

**WORKING WASH BUFFER**

Dilute the 20X wash buffer concentrate with deionized or distilled water 1:20. For example, 5 ml of wash buffer concentrate should be diluted to a total volume of 100 mL with deionized or distilled water.

**STABILITY OF OPENED KIT COMPONENTS AND DILUTED REAGENTS**

The diluted wash buffer is stable for at least one week when stored at room temperature. Substrate is stable for the expiration date of the kit. The micro titer plates should be opened after they have been kept at room temperature for 20-30 minutes. After removing the required number of strips, the plates should be resealed in the foil pouch bags along with the desiccant and stored at 2°-8°C.

**ASSAY PROCEDURE:**

1. Dispense 100µl of specimen diluent into individual test wells.
2. Dispense 100µl positive control and negative control duplicate into individual wells.
3. Add 10µl of each test sample into duplicate test wells; vortex to mix.
4. Incubate for 30 minutes at 37°C

5. Wash each well 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
6. Add 100µl of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 minutes. Do not add Enzyme Conjugate to the blank well.
7. Incubate for 20 minutes at 37°C
8. Wash the plate 5 times as step 6.
9. Add one drop (50µl) of Substrate Solution A (HRP-substrate) to each well, then add one drop (50µl) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 minutes. .
10. Add one drop (50µl) of Stop Solution to each well to stop the color reaction. Read O.D. at 450 nm with an EIA reader.

**RESULT INTERPRETATION**

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells, The positive control OD value should be ⩾0.8, the negative control should be ⩽0.10 ):

Cut-off Calculations:
Take average OD values of Negative control and add 0.15:

**1x NC + 0.15 = Cut-off.**

Positive OD reading: ⩾Cut-off value
Negative OD reading: < Cut-off value

**LIMITATIONS OF THE PROCEDURE**

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognised methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5 %) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25°C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, and they should not be mixed with one another.
- All reagents have to be used within the expiry period.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

**REFERENCES**


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