# Human Tetanus Toxoid antibody ELISA Kit

*Cat.No: DEIA1026*

*Lot. No. (See product label)*

## Size

96T

## Intended use

This kit is an indirect enzyme-linked immunosorbent assay for quantitative detection of IgG antibodies to Tetanus Toxoid in human serum or plasma. It is intended for diagnosing and monitoring of patients related to infection by Tetanus Toxoid.

## Principle Of The Test

The quantitative immunoenzymatic determination of IgG antibodies against tetanus toxin is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with tetanus toxin antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material, horseradish peroxidase (HRP) conjugate is added. This conjugate binds to antigen-antibody complexes. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of tetanus toxin-specific IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

## Reagents And Materials Provided

1. Human Tetanus toxin Ab Coated Wells, 96-well polystyrene microplate (12 strips of 8 wells): 1 plate, Ready-to-use.
2. Human Tetanus IgG Standard 0 IU/mL: 1 mL/vial, 1 vial, Ready-to-use.
3. Human Tetanus IgG Standard 0.01 IU/mL: 1 mL/vial, 1 vial, Ready-to-use.
4. Human Tetanus IgG Standard 0.02 IU/mL: 1 mL/vial, 1 vial, Ready-to-use.
5. Human Tetanus IgG Standard 0.04 IU/mL: 1 mL/vial, 1 vial, Ready-to-use.
6. Human Tetanus IgG Standard 0.06 IU/mL: 1 mL/vial, 1 vial, Ready-to-use.
7. Human Tetanus IgG Standard 0.10 IU/mL: 1 mL/vial, 1 vial, Ready-to-use.
8. HRP-conjugate reagent: 1 bottle, 10 mL, Ready-to-use.
9. Sample Diluent A: 1 bottle, 10 mL, Ready-to-use.
10. Sample Diluent B: 1 bottle, 10 mL, Ready-to-use.
11. Dilution plate (uncoated plate), 96-well polystyrene microplate: 1 plate, Ready-to-use.
12. CHROMOGEN SOLUTION A: 1 vial, 6 mL, Ready-to-use.
13. CHROMOGEN SOLUTION B: 1 vial, 6 mL, Ready-to-use.
14. Wash Solution Concentrate (20×), 50 mL with preservatives: 1 bottle
Final concentration: dilute 1+19 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.
15. Stop Solution: 1 bottle, 6 mL, Ready-to-use.
16. Datasheet: 1
17. Plastic Bag: Resealable, for the dry storage of non-used strips
18. Plastic Foils: 3 pieces to cover the microtiter strips during the incubation


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## Materials Required But Not Supplied

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5°C.
8. Microshaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.

## Storage

The components of the kit will remain stable (12 months) through the expiration date indicated on the label and package with desiccant when stored between 2-8°C, do not freeze. To assure maximum performance of this Tetanus toxin IgG ELISA kit, protect the reagents from contamination with microorganism or chemicals during storage.

## Specimen Collection And Preparation

Principally serum or plasma (EDTA, heparin, citrate) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (4-8°C) for up to 48 hours, for a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results. For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

## Assay Procedure

Bring all reagents and samples to room temperature before use.

1. Dilute Concentrated(20x) Wash Solution with distilled water/deionzied water ready for use.

2. Prepare sufficient amount of microtiter wells for the standards (6 wells: 0、0.01、0.02、0.04、0.06、0.10IU/ml), and samples in duplicate as well as for one substrate blank.

3. Mix 10ul sample with 100ul Sample Diluent A in each well of sample dilution plate to get prediluted sample.

4. Mix 10ul prediluted sample with Sample Diluent B in each well(except 6 standard wells&one blank well) of coated ELISA plate, then add 6 standards to the wells without sample dilution.

5.Cover plate with the enclosed foil and incubate at 37°C for 30 minutes.

6. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally five times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

7. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.


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8. Cover plate with the enclosed foil and incubate at 37°C for 30 minutes.

9. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally five times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

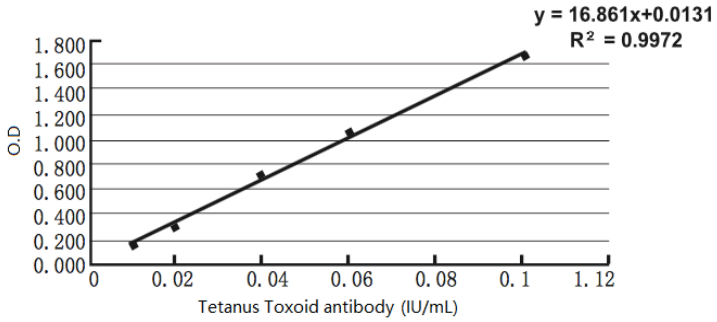
10. Pipet 50µL CHROMOGEN SOLUTION A and 50µL CHROMOGEN SOLUTION B into the wells. This time also the substrate blank is pipetted. Cover plate with the enclosed foil and incubate at 37°C for 15 minutes in the dark (e.g. drawer).

11. To terminate the substrate reaction, pipet 50 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm

## Typical Standard Curve

This standard curve is provided for demonstration only. A standard curve should be generated for each assay plate.

| **Standards** (IU/ml) | 0.01 | 0.02 | 0.04 | 0.06 | 0.1 | **Sample** |
|---|---|---|---|---|---|---|
| **OD**450nm | 0.158 | 0.333 | 0.726 | 1.056 | 1.671 | 1.001 |




## Detection Range

Detection Range: 0.01IU/ml~0.1IU/ml

In order for an assay to be considered valid (R2 ≥ 0.98), the following criteria must be met:
Standard A (0 IU/ml): Absorbance value ≤ 0.10
Standard A (0.1 IU/ml): Absorbance value ≥ 0.80

If the sample OD is higher than the upper limit of standard curve, the sample should be re-diluted and the experiment rerun. Multiply the result by dilution factor when calculating the unknown.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## Precautions

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.
1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. CAUTION - CRITICAL STEP: Allow the reagents and samples to stabilize at room temperature (18-30°C ) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37°C inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1 hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M $H_2SO_4$ ) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. Proclin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.


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