

Version 1b Last updated 20 March 2026

# **ab247204**

## ***Clostridium tetani* toxin**

### **5S IgG plus ELISA kit**

For the quantitative determination of IgG class antibodies against *Clostridium tetani* toxin 5S in human serum or plasma (citrate, heparin).

This product is for research use only and is not intended for diagnostic use.

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# 1. Overview

*Clostridium tetani* toxin 5S IgG plus ELISA kit (ab247204) is designed for the quantitative determination of IgG class antibodies against *Clostridium tetani* toxin 5S antigens in human serum or plasma (citrate, heparin).

The quantitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. 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 specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

Clostridia are spore-forming gram-positive bacteria. The round spores are built at the terminal end which results in the microscope in a "tennis racket" like shape. *Clostridium tetani* is ubiquitously present in the soil and intestine of humans and animals. Ingestion of bacteria or growth in the intestine of man or animal is without harm. The spores are extremely resistant towards heat and can stay infectious for a long period. The bacteria can get under the skin by even smallest wounds. In Europe tetanus mainly occurs after injuries and sometimes postoperative, whereas in developing countries Tetanus is widely disseminated. The WHO assumes that one million people die because of tetanus worldwide per year.

Tetanus toxin is an excellent immunogen in man - only one antigenic type of toxin. The only effective way to control tetanus is by prophylactic active immunization.

## 2. Protocol Summary

Prepare all reagents, samples, standards and controls as instructed



Add 100  $\mu$ L control, standard or sample to appropriate wells



Incubate for 1 hour at 37°C



Aspirate and wash each well three times with 300  $\mu$ L 1X Washing  
Solution



Add 100  $\mu$ L of HRP conjugate to each well. Incubate for 30 minutes  
at room temperature. Repeat the washing steps.



Add 100  $\mu$ L TMB Substrate Solution to each well and incubate for 30  
minutes at room temperature.



Add 100  $\mu$ L Stop Solution and read OD at 450/620 nm within 30  
minutes after addition of the stop solution.

### 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website <https://www.abcam.com/en-us>.

### 4. Storage and Stability

**Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

| Item                                               | Quantity | Storage Condition |
|----------------------------------------------------|----------|-------------------|
| 20X Washing Solution                               | 50 mL    | +4°C              |
| Cover Foil                                         | 1 unit   | +4°C              |
| Stop Solution                                      | 15 mL    | +4°C              |
| TMB Substrate Solution                             | 15 mL    | +4°C              |
| Clostridium tetani toxin Coated Microplate (IgG)   | 1 unit   | +4°C              |
| anti-human IgG HRP conjugate                       | 20 mL    | +4°C              |
| Clostridium tetani toxin 5S IgG Control            | 2 mL     | +4°C              |
| IgG Sample Diluent                                 | 100 mL   | +4°C              |
| Clostridium tetani toxin IgG Standard A – 0 IU/mL  | 2 mL     | +4°C              |
| Clostridium tetani toxin IgG Standard B – 10 IU/mL | 2 mL     | +4°C              |
| Clostridium tetani toxin IgG Standard C – 25 IU/mL | 2 mL     | +4°C              |
| Clostridium tetani toxin IgG Standard D – 50 IU/mL | 2 mL     | +4°C              |
| Clostridium tetani toxin IgG Standard E – 50 IU/mL | 2 mL     | +4°C              |

The standards are calibrated in accordance with the "1st WHO International Standard Pertussis antiserum (human)", Code 06/140, of the National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK.

## **7. Materials Required, Not Supplied**

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450/620 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Plate shaker for all incubation steps.

## **8. Technical Hints**

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.

## 9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

### 9.1 20X Washing Solution:

Prepare 1X Washing Solution by diluting 20X Washing Solution with deionized water. To make 200 mL combine 10 mL 20X with 190 mL deionized water. Mix thoroughly and gently. The diluted buffer is stable for 5 days at room temperature. In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

#### **Clostridium tetani toxin Coated Microplate (IgG):**

The break-apart snap-off strips are coated with Clostridium tetani toxin antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 4 °C.



## 10. Sample Preparation

- Use human serum or plasma (citrate, heparin) samples with this assay.
- If the assay is performed within 5 days after sample collection, the samples should be kept at 4°C; otherwise they should be aliquoted and stored deep-frozen (-70°C). If samples are stored frozen, mix thawed samples well before testing.
- Avoid repeated freezing and thawing.
- Heat inactivation of samples is not recommended.

## 11. Sample Dilution

- Before assaying, all samples should be diluted 1:100 with IgG Sample Diluent. Dispense 10 µL sample and 990 µL IgG Sample Diluent into tubes to obtain a 1:100 dilution and thoroughly mix with a Vortex.

## 12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
  - We recommend that you assay all controls and samples in duplicate.
- 
- 12.1** Prepare all reagents, controls and samples as directed in the previous sections.
  - 12.2** Use the plate layout to plan the location for all controls and samples.
  - 12.3** Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
  - 12.4** Add 100 µL of all diluted samples and controls to appropriate wells. Leave well A1 as the Substrate blank. Cover the wells with the cover foil.
  - 12.5** Incubate for 1 hour at 37°C.
  - 12.6** When incubation is completed, remove the foil, aspirate the content of the wells and wash each well 3 x 300 µL with 1X Washing Solution. Avoid overflows from the reaction wells. The interval between washing and aspiration should be less than 5 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.
  - 12.7** Add 100 µL of HRP conjugate to each well, except A1, and incubate for 30 minutes in the dark at room temperature.
  - 12.8** Repeat the washing steps as per 12.6.
  - 12.9** Add 100 µL of TMB Substrate Solution to each well. Incubate for exactly 30 minutes at room temperature in the dark. A blue color occurs due to an enzymatic reaction.
  - 12.10** Add 100 µL of Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a color change from blue to yellow occurs.
  - 12.11** Measure the absorbance at 450/620 nm within 30 minutes after addition of the Stop Solution.

## 13.Measurement

**13.1** Adjust the microplate reader to zero using the Substrate blank.

**Δ Note:** If due to technical reasons that the microplate reader cannot be adjusted to zero using the blank, then subtract its absorbance value from all other absorbance values measured to obtain reliable results.

**13.2** Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and sample in the plate layout.

**13.3** Biochromatic measurement using a reference wavelength of 620 nm is recommended.

**13.4** Where applicable calculate the mean absorbance values for all duplicates.

**13.5** Run validation criteria:

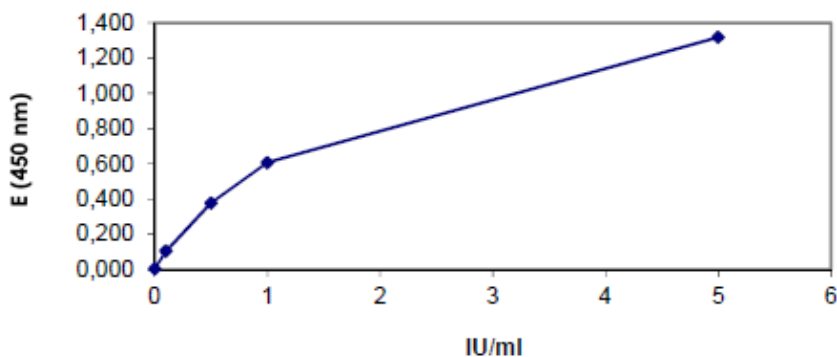
- For an assay to be considered valid, the following criteria must be met.
- If these criteria are not met, the test is not valid and must be repeated.

| Controls        | Absorbance value                                    |
|-----------------|-----------------------------------------------------|
| Substrate Blank | < 0.1                                               |
| Standard A      | < 0.2                                               |
| Standard B      | > 0.05                                              |
| Standard C      | > Standard B                                        |
| Standard D      | > Standard C                                        |
| Standard E      | > 0.8                                               |
| Control         | Result in IU/mL within range indicated on the label |

Standard A < Standard B < Standard C < Standard D < Standard E

## 14. Calculations

- 14.1** To obtain quantitative results in IU/mL plot the mean absorbance values of the 5 Standards A - E on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0, 0.1, 0.5, 1.0 and 5.0 IU/mL) and draw a standard curve (absorbance values on the y-axis, concentrations on the x-axis).
- 14.2** Read results from this calibration curve employing the mean absorbance values of each patient sample and control.
- 14.3** For the calculation of the standard-curve, the mathematical Point to Point function should be used.



**Figure 1.** Typical Standard Curve.

## 15. Interpretation of Results

| Concentration    | Interpretation of results                                                                                                                                                                                                                 |
|------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| < 0.1 IU/mL      | No protective antibody level, or no reliable protection. Immediate full course of basic immunization or booster injection and control of antibody concentration 4 to 6 weeks later is recommended.                                        |
| 0.11 – 0.5 IU/mL | Reliable protection. Booster injection and control of antibody concentration 4 to 6 weeks later is recommended.                                                                                                                           |
| 0.51 – 1.0 IU/mL | Reliable protection; control of antibody concentration after about 2 years is recommended. Booster injection is not required.<br><br>Note: In cases of antibody concentrations greater than 0.5 IU/mL vaccination can cause side effects! |
| 1.1 – 5.0 IU/mL  | Range of long term protection: Control after 5 to 10 years.                                                                                                                                                                               |
| > 5.0 IU/mL      | Range of long term protection: Control after 10 years immunisation and to record the data on the certificate of vaccination.                                                                                                              |

Diagnosis of an infectious disease should not be established based on a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.

## 16. Typical Sample Values

### PRECISION –

- The reproducibility of the kit was determined by comparing a minimum of 24 replicates of 3 different samples in one assay (intra-assay) and by comparing 3 different samples assayed in 12 different runs (inter-assay).
- Acceptance Criterion: CV < 15 %

|                  | Intra-Assay |       |       | Inter-Assay |       |       |
|------------------|-------------|-------|-------|-------------|-------|-------|
|                  | 1           | 2     | 3     | 1           | 2     | 3     |
| <b>n =</b>       | 24          | 24    | 24    | 12          | 12    | 12    |
| <b>Mean (OD)</b> | 1.306       | 1.805 | 1.591 | 0.06        | 0.084 | 0.658 |
| <b>CV (%)</b>    | 3.6         | 3.46  | 5.34  | 9.62        | 11.33 | 13.99 |

### DIAGNOSTIC SPECIFICITY –

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

It is 100% (95% confidence interval: 76.84% - 100%).

### DIAGNOSTIC SENSITIVITY –

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

It is 99.22% (95% confidence interval: 95.76% - 99.98%).

### ANALYTICAL SENSITIVITY –

The analytical sensitivity (according to CLSI EP17-A) is defined as the apparent concentration of the analyte that can be distinguished from the zero calibrator. It is 0.02 IU/mL.

### INTERFERENCES –

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

## **CROSS REACTIVITY –**

Investigation of a sample panel of 9 specimens from patients with confirmed diseases other than Tetanus was tested to establish the analytical specificity. The specimens were from patients infected with pathogens that may cause similar signs and symptoms to those observed for Tetanus or from individuals with diseases or conditions that have the potential for cross-reactivity.

The pathogens tested were *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, *Bordetella pertussis* and *Borrelia*.

## **MEASUREMENT RANGE –**

The measurement range is 0.02 IU/mL – 5.0 IU/mL.

It is defined by the analytical sensitivity (Limit of Detection: 0.02 IU/mL) and the concentration of the highest standard (5.0 IU/mL).

## **LIMITATIONS OF THE PROCEDURE –**

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

## **Analytical Sensitivity (Limit of Detection) –**

The terms Limit of Blank (LoB) and Limit of Detection (LoD) are used to describe the smallest concentration that can be reliably measured by an analytical procedure. The LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. The LoD is the lowest analyte concentration likely to be reliably distinguished from the LoB and at which detection is feasible.

The LoD or analytical sensitivity was determined according to the approved guideline CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation".

For this the zero standard, Standard A (= blank) and one low concentration sample were determined 60-fold on the *Clostridium tetani* toxin IgG ELISA.

The LoD was calculated according to the following formula:

$$\begin{aligned}\text{LoD} &= \text{LoB} + 1.645 \times \text{SD}_{\text{low concentration sample}} = \\ &= \text{mean}_{\text{blank}} + 1.645 \times \text{SD}_{\text{blank}} + 1.645 \times \text{SD}_{\text{low concentration sample}}\end{aligned}$$

Specification: Acceptance criterion =  $\text{LoD} \leq 0.05 \text{ IU/mL}$

Results:

The Limit of Detection (LoD) was estimated at 0.02 IU/ml. Therefore, the acceptance criterion is met.



## 17. Notes

## Technical Support

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