



**User's Manual**

# **Rabbit Anti-Tetanus Toxoid IgG ELISA Kit**



**DEIASL273**



**96T**



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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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## PRODUCT INFORMATION

### Intended Use

This kit is intended to detect anti-Tetanus Toxoid IgG antibody in rabbit serum post vaccination.

### General Description

Tetanus also called lockjaw is caused by neurotoxin from deep wound infection with *Clostridium tetani*. Symptoms include muscle spasms of the jaw and elsewhere in the body. These symptoms are caused by a toxin released from the bacteria. Vaccines have been developed with Tetanus Toxoid (inactivated Tetanus toxin) to protect from infection. Antibodies should be detectable in animals post vaccination.

### Principles of Testing

Microtiter wells coated with Tetanus Toxoid are exposed to test specimens, which may contain Rabbit IgG reactive antibodies. After an incubation period, unbound components in the test sample are washed away. Specific bound Rabbit IgG react with an anti-Rabbit IgG conjugated with HRP during a second incubation period. Following a second wash cycle, specific bound enzyme conjugate is detected by reaction with the substrate solution, 2,2' Azino-di[3-ethyl-benzthiazolinesulfonate] (ABTS). The assay is measured spectrophotometrically to indicate the level of Rabbit Anti-Tetanus Toxoid antibodies present in a sample.

### Reagents And Materials Provided

1. Coated microwell strips. Plastic microtiter wells coated with Tetanus Toxoid in foil pouch with desiccant. 1 plate (96 wells)
2. Rabbit Anti-Tetanus Toxoid Positive Calibrator 10000 U/ml 0.4 mL
3. Sample Diluent. 30 mL
4. Conjugate. Anti-rabbit IgG conjugated to horseradish peroxidase enzyme containing 0.01% Bromonitrodioxane as preservative. 12 mL
5. Wash Buffer (20x concentrated). Tris buffered saline pH 7.8-8.0, containing 0.05% Tween 20. Must be diluted before use. 1 Bottles 60 mL
6. Substrate Solution. Ready to use. 2,2' Azino-di[3-ethyl-benzthiazolinesulfonate] ABTS. 12 mL
7. Stop Solution. 1.25% sodium fluoride. 5 mL

### Materials Required But Not Supplied

1. Disposable tip micropipettes to deliver volumes of 5  $\mu$ L, 10  $\mu$ L, 25  $\mu$ L, 100  $\mu$ L and 200  $\mu$ L (multichannel pipette preferred for dispensing reagents into microtiter plates).
2. Distilled or deionized water.
3. 37 ( $\pm$ 1) $^{\circ}$  C incubator.
4. Clean, disposable plastic/ glass test tubes, approximate capacities 5 mL and 10 mL.

5. Range of standard, clean volumetric laboratory glassware consisting of, at least, 15 mL and 100 mL beakers, 1 L graduated cylinder, 1 mL, 5 mL, and 10 mL glass pipettes.
6. Absorbent paper towels.
7. Automatic microtitration plate washer or laboratory wash bottle.
8. Microtitration plate reader with 450 nm filter.
9. Latex gloves, safety glasses and other appropriate protective garments.
10. Biohazard infectious waste containers.
11. Safety pipetting devices for 1 mL or larger pipettes.
12. Timer.

#### Automatic, or Semi-automatic Processing

The Rabbit Anti-Tetanus Toxoid Assay may be used with a variety of automatic or semi-automatic processors/liquid handling systems. It is essential that any such system is qualified, before it is used routinely, by demonstrating that the Rabbit Anti-Tetanus Toxoid Assay results obtained using the automatic processor are equivalent to those obtained for the same specimens using the manual test method. Subsequently the automatic processor should be periodically re-qualified.

## Storage

All reagents should be stored at 2-8°C, and should not be used beyond the expiration date on the label. Once opened, microtitration strips may be stored at 2-8°C until the expiration date on the label, provided that desiccated conditions are maintained. Unused strips should be returned to their original foil pouch along with the sachet of desiccant. Opened pouches should be securely resealed by folding over the open end and securing it with adhesive tape.

The working strength Wash Buffer should not be stored for longer than 3 weeks at 2-8°C. It is recommended that Wash buffer be freshly diluted before each assay. If the working strength buffer becomes visibly cloudy or develops precipitate during the 3 weeks, do not use it.

#### Indications of Deterioration

The Rabbit Anti-Tetanus Toxoid Assay may be considered to have deteriorated if:

1. The kit fails to meet the required criteria for a valid test (see Quality Control).
2. Reagents becoming visibly cloudy or develop precipitate. Note: Concentrated Wash buffer, when cold, normally develops crystalline precipitates, which re-dissolve on heating at 37°C.
3. The Substrate Solution turns dark green. This is likely to be caused by contamination of the Substrate Solution.

## Specimen Collection And Preparation

Obtain blood and allow clot to form. Remove serum from clot. Insoluble materials should be removed by centrifugation. Remove the serum aseptically. Serum samples should be refrigerated as soon as possible after collection. If not assayed within 48 hours, the samples should be aliquotted and frozen. Avoid repeated freezing/thawing of samples. Samples should not contain sodium azide.

## Reagent Preparation

## 1. Positive Anti-Tetanus Toxoid Calibrator 10000 U/ml

- a. Prepare working strength calibrator by diluting 50ul of the positive Anti-Tetanus Toxoid calibrator into 450 ul (1:10 dilution) of sample diluent. This will give a final concentration of 1000 U/ml.
- b. Prepare six serial two fold dilutions (250ul standard with 250ul sample diluent) to prepare 500 U/ml, 250 U/ml, 125 U/ml, 62.5 U/ml, 31.2 U/ml and 15.6 U/ml calibrators using the sample diluent. Each calibrator plus a sample diluent (0 U/ml) should be run in duplicate.

## 2. Wash Buffer

Prepare working-strength Wash buffer by diluting 1 part concentrate with 19 parts of distilled or de-ionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficient for immediate needs. Each row of 8 wells may be adequately washed with 150 mL of working strength buffer.

## Assay Procedure

### Wash Cycle

Efficient washing to remove un-complexed components is a fundamental requirement of enzyme immunoassay procedures. The Rabbit Anti-Tetanus Toxoid assay utilises two standard five-wash cycles. Automatic plate washers may be used provided they meet the following criteria:

1. All wells are completely aspirated.
2. All wells are filled to the rim (350 µL) during the rinse cycle.
3. Wash buffer is dispensed at a good flow rate.
4. The plate washer must be well maintained to prevent contamination from previous use. Manufacturer's cleaning procedures must be followed diligently.

For each wash cycle the machine should be set to five consecutive washes. On completion of the cycle, invert the microtitration plate and tap firmly on absorbent paper towels. Check for any residual wash buffer in the wells and blot dry the upper surface of the wells with a paper towel.

Alternatively, the following manual system may be employed:

1. Aspirate well contents using a vacuum line fitted with a trap.
2. Fill all wells to the brim with wash buffer dispensed from a squeeze-type laboratory wash bottle.
3. Aspirate all wells.
4. Repeat steps 2 and 3, four times for a total of five cycles.
5. Invert the microtiter plate and tap firmly on absorbent paper towels.

### Assay Steps

1. Allow all reagents to reach room temperature (18-25°C).
2. Each Rabbit serum to be tested should be diluted to a 1:50 dilution. Dilute the serum 1:50 in Sample Diluent. For example: add 5 ul of serum sample to 245 ul of 1X Sample Diluent. If not assayed immediately, diluted samples should be stored at -20°C or below.
3. Select sufficient microtiter well strips to accommodate all test specimens and the 7 calibrators run in duplicate (including sample diluent). Fit the strips into the holding frame. Label wells according to specimen identity using the letter/number cross-reference system molded into the plastic frame.

4. Dispense 100  $\mu$ L of each specimen, calibrator and sample diluent into appropriate wells. Note: All standards and samples should be tested in duplicate.
5. Incubate at 37( $\pm$ 1) $^{\circ}$ C for 45 ( $\pm$ 5) minutes.
6. Aspirate the contents of the wells and wash the microtiter plate as described in the Rinse Cycle section.
7. Pipette 100  $\mu$ L of anti-IgG conjugate into each well and incubate at 37( $\pm$ 1) $^{\circ}$ C for 45 ( $\pm$ 5) minutes.
8. Aspirate the conjugate from the wells and wash the microtiter plate as described in the Wash section.
9. Without delay, dispense 100  $\mu$ L Substrate Solution into each well. A multichannel pipette should be used for best results. Leave at room temperature (18-25 $^{\circ}$ C) protected from direct sunlight, for 30 ( $\pm$ 2) minutes.
10. If the plate is not read immediately, pipette 25  $\mu$ L of Stop Solution into each test well. Within 15 minutes, read the plate at 405 nm using a microtiter plate reader blanked on the sample diluent well. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.

## Quality Control

The following criteria should be met for a valid assay:

The 0 U/ml standard (sample diluent) should be  $\leq 0.10$

The 1000 U/ml standard should be  $\geq 0.60$

## Calculation

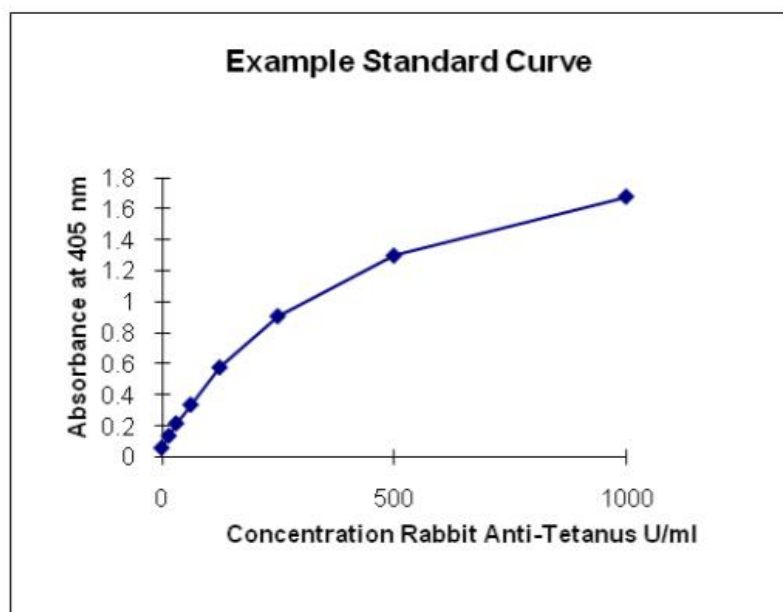
Manual Method: The calibration curve can be constructed manually on linear graph paper.

1. Calculate the mean absorbance for each standard.
2. Plot the mean absorbance on the y-axis versus the concentration of the standard on the x-axis. Connect the points to produce a point to point curve. Do not force the line to be linear.
3. The concentration of the specimens can be found directly from the standard curve. Please note that the standard curve has been standardized to the 1:50 serum dilution. The final U/ml can be read directly from the curve with no factor for dilution if the sample was assayed at a 1:50 dilution.

## Typical Standard Curve

Table 1. Example Data at 405nm.

Sample	405 nm abs.	Mean abs.	U/mL
Sample Diluent (0 U/mL)	0.06		
"	0.06	0.06	
Standard 7 (15.6 U/mL)	0.13		
"	0.15	0.14	
Standard 6 (31.2 U/mL)	0.22		
"	0.22	0.22	
Standard 5 (62.5 U/mL)	0.32		
"	0.36	0.34	
Standard 4 (125 U/mL)	0.56		
"	0.60	0.58	
Standard 3 (250 U/mL)	0.88		
"	0.94	0.91	
Standard 2 (500 U/mL)	1.26		
"	1.34	1.30	
Standard 1 (1000 U/mL)	1.64		
"	1.72	1.68	
Specimen #1	0.42		
"	0.44	0.43	86
Specimen #2	0.67		
"	0.73	0.70	221



Note: The above standard curve is only an example and should not be used to generate any results.

Computer-Assisted Method: Computer assisted data reduction may be used to create the standard curve. Software providing a point to point curve fitting routine provides acceptable results.

Procedure for Samples with Rabbit Anti-Tetanus Toxoid assay values greater than the highest standard.

In order to obtain accurate results for samples with Rabbit Anti-Tetanus Toxoid assay values greater than the highest standard it is necessary to dilute and re-test the sample. Diluting the serum specimen 10-fold is recommended. For example: Make a 10-fold dilution by adding 0.1 mL of the initial specimen dilution (1:50) to 0.90 mL of sample diluent. Mix thoroughly and repeat the assay according to the Assay Procedure. Multiply the results by 10 to determine the correct Rabbit Anti-Tetanus Toxoid assay values in the sample.

## Precautions

1. This kit should be used in strict accordance with the instructions in the Package Insert.
2. Do not use Rabbit Anti-Tetanus Toxoid Assay kits after the expiration date printed on the outer carton label.
3. Do not cross contaminate reagents. Always use fresh pipette tips when drawing from stock reagent bottles.
4. Always use clean, preferably disposable, glassware for all reagent preparation.
5. Allow foil bags to warm to room temperature before opening. This avoids condensation on the inner surface of the bag, which may contribute to a deterioration of coated strips intended for future use.
6. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. Follow manufacturer's recommendations for automatic processors.
7. Always keep the upper surface of the microtiter strips free from excess fluid droplets. Reagents and buffer over-spill should be blotted dry on completion of the manipulation.
8. Do not allow the wells to completely dry during an assay.
9. Disposal or decontamination of fluid in the waste reservoir from either the plate washer or trap for vacuum line in the manual system should be in accordance with guidelines set forth in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.
10. Automatic or semi-automatic ELISA processors or liquid handling systems should be qualified specifically for use with Rabbit Anti-Tetanus Toxoid Assay by demonstration of equivalence to the manual processing methods.
11. Consistent with good laboratory practice, it is recommended that all pipetting devices (manual or automatic), timers and thermometers are regularly calibrated according to the manufacturer's instructions.
12. Care must be taken to ensure that specimens are dispensed correctly to each test well. If a specimen is inadvertently not added to a well, the result for that well will be non-reactive, regardless of the actual result of the specimen.

## Limitations

1. Assay values determined using assays from different manufacturers or different methods may not be used interchangeably. PLEASE NOTE: There is not a international standard for Rabbit Anti-Tetanus Toxoid, therefore the units are assigned arbitrarily. Units between manufactures may not be the same.
2. Samples with very high Rabbit Anti-Tetanus Toxoid assay values levels may exhibit in a prozone effect.
3. The assay cannot be used to quantitate samples with Rabbit Anti-Tetanus Toxoid assay values greater than the highest standard without further serial dilution of the samples. See the Interpretation of Results section for directions on testing such samples.
4. The performance characteristics have not been established for any matrices other than serum.

