HUMAN ANTI-TETANUS TOXOID IMMUNOGLOBULIN LIQUID REAGENT KIT for use on SPA$_{PLUS}$™

For In-Vitro Research Use Only

Product Code: LK710.S.U

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1 INTENDED USE

This kit is intended for the quantitative *in vitro* measurement of specific antibodies against Tetanus toxoid in heparinised or EDTA human plasma, using the Binding Site SPA$_{PLUS}$ turbidimetric analyser.

2 SUMMARY AND EXPLANATION

Anti-tetanus toxoid antibodies are raised in response to vaccination with Tetanus toxoid protein. A patient's response to the immunisation may be assessed, subsequently, by the serological determination of their anti-tetanus toxoid antibody levels using this quantitative turbidimetric method.

3 PRINCIPLE

The determination of soluble antigen concentration by turbidimetric methods involves the reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed a portion of the light is transmitted and focused onto a photodiode by an optical lens system. The amount of transmitted light is indirectly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

Latex-enhanced Antigens Some antigen-antibody reactions do not form sufficiently large immune complexes to be detected turbidimetrically. If the antigen is coated onto latex particles of a suitable size, the light scattering ability of the immune complexes formed with antibody is enhanced sufficiently to enable turbidimetric detection.

4 REAGENTS

4.1 Latex reagent to a-Tetanus toxoid immunoglobulin. Latex particles coated with inactivated Tetanus toxoid antigen. Supplied in stabilised liquid form. The reagent contains 0.033% sodium azide, 0.1% E-amino-n-caproic acid (EACA), 0.01% benzamidine and 0.05% ProClin™ as preservatives.

4.2 Calibrators and Controls. These consist of pooled human serum and are supplied in stabilised liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. The assay is calibrated against the 1st International Standard for Tetanus Immunoglobulin TE-3, supplied by the National Institute for Biological Standards and Control (NIBSC; www.nibsc.ac.uk).

4.3 Supplementary Reagent (Reaction Buffer). This contains 0.099% sodium azide as a preservative.

**ProClin™ is a trademark of Rohm and Haas Corp., Philadelphia, PA.*

5 CAUTION

All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either approved by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and ProClin 300 and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times. Results are likely to be invalid if parameters other than those stated in these instructions are used.

Reagents from different batch numbers of kits are **NOT** interchangeable. If large numbers of tests are performed care should be taken to ensure that all the reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kit should be stored at 2-8°C and can be used until the expiry date shown on the kit box label. DO NOT FREEZE. The latex reagent, calibrator, and control may be stored for up to three months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator.
The Latex Reagent and Supplementary Reagent (Reaction Buffer) may be stored, uncapped, on the analyser for up to 30 days, provided that the main power switch (located at the rear of the left hand panel) is left switched on.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen plasma samples. Blood samples should be collected by venepuncture, into EDTA or heparin blood collection tubes The plasma may be stored at 2-8°C for up to 14 days prior to assay, or for prolonged storage kept undiluted at -20°C or below. Repeated freezing and thawing should be avoided.
Microbially contaminated, haemolysed and lipaemic plasma, and samples containing particulate matter should not be used.

8 METHODOLOGY

8.1 Materials Provided

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8.1.1 5 x 200 Tests *Human Tetanus SPA$_{PLUS}$* toxoid *Latex Reagent.*
8.1.2 5 x 200 Tests *Tetanus toxoid SPA$_{PLUS}$ Reaction Buffer*
8.1.3 1 x *Human Tetanus toxoid SPA$_{PLUS}$ Calibrator Set 1-6 (6 x 1.0mL)*
8.1.4 1 x 1.5mL *Human Tetanus toxoid SPA$_{PLUS}$ Low Control*
8.1.5 1 x 1.5mL *Human Tetanus toxoid SPA$_{PLUS}$ Control*

8.2 Materials required but not provided

8.2.1 Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
8.2.2 A fully operational and equipped SPA$_{PLUS}$ analyser.
8.2.3 Sample Diluent, 99: Dil 1 Pack Code: SN080.S

8.3 Reagent preparation

Before loading, gently mix by inversion ensuring no foam or bubbles are generated or remain on the surface as these may interfere with reagent aspiration.

8.4 Test procedure

The user should be familiar with the operation of the SPA$_{PLUS}$ analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the manufacturer's instructions and the assay protocol entered as described below.

8.4.1 Programming parameters

8.4.1.1 Select **Item** from the main screen.
8.4.1.2 Select the correct item number (T.Tox = 13,) and click **Edit** to open page 1 of 4.
8.4.1.3 Enter the parameters as below.

Item Name 13 T.Tox

DATA INFORMATION
Units IU/mL
Decimals 2

ANALYSIS
Type End ▼
Main W.Length 1 600 ▼
Sub W.Length ▼
Method

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION
Type Logit 2 ▼
Standard
1 # 4 #
2 # 5 #
3 # 6 #

NORMAL RANGE

	MALE LOW	MALE HIGH	FEMALE LOW	FEMALE HIGH
Serum	[ ]	[ ]	[ ]	[ ]
Urine	[ ]	[ ]	[ ]	[ ]
Plasma	[ ]	[ ]	[ ]	[ ]
CSF	[ ]	[ ]	[ ]	[ ]
Dialysis	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]

Page : 1 Print Hard Copy Next Page Save Return

Item Name 13 T.Tox

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 9.5
REAGENT1 VOL 165 µL
REAGENT2 VOL 80

Third mix ● **OFF** ○ **ON**
R1 Blank ● **Water – Blank** ○ **R1 – Blank1**

MONITOR
0 LEVEL SPAN 1
SPAN 3

DATA PROCESS
READ **ABSORBANCE LIMIT**
START END
MAIN 53 54 LOW -3
SUB 35 36 HIGH 3

FACTOR **Reaction Check**
Blank correction 1 ○ ON ● OFF
ENDPOINT LIMIT 2 CHECK POINT
LINEAR CHECK (%) 0 LOW -3
HIGH 3

DILUTION
Diluent ● 99: Dil 1 ○ 100: Dil 2
Pre Dilution Rate 10 ▼
Auto Rerun Dilution Rate ▼

PROZONE CHECK

	START	END	LIMIT (%)	
FIRST	[ ]	[ ]		
SECOND	[ ]	[ ]	[ ]	● Low ○ High
THIRD	[ ]	[ ]	[ ]	● Low ○ High

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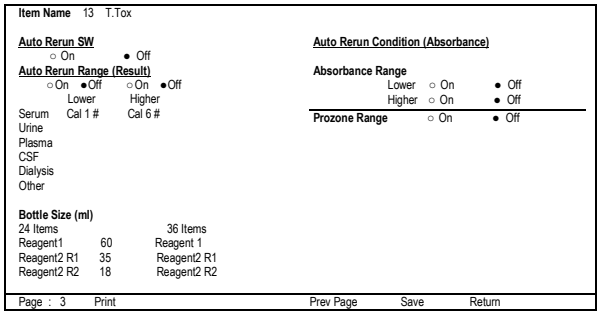

N.B. The calibrator (Standard #) values to be entered on page 1 are found on the Quality Control Certificate and should be entered in ascending order, i.e. lowest value first. **IMPORTANT:** the analyser will automatically calculate and enter the correct measuring ranges on Item pages 3 and 4 providing **the ENTER button is pressed after typing the value for calibrator 6 on page 1**. You may view Item parameter page 4 to ensure correct value entry.

8.4.1.4 Click **Save** once all parameters have been entered and updated.

8.4.2 Reagent loading and test registration

8.4.2.1 Place reagent bottles into an empty position on the reagent carousel. Note the reagent position number.
8.4.2.2 Click onto **Bottle** from the Main Screen
8.4.2.3 Enter the reagent position into the test position box. Click **Bottle Read**. The number of tests will automatically be entered as 200.
8.4.2.4 The same should also be done for the sample diluent, which will register 200 tests once read.
8.4.2.5 Click **Update** to save changes.

8.4.3 Setting up controls

8.4.3.1 Click **System** from the main screen, and then select **Sys Para.**
8.4.3.2 Check that '**by Item'** in the run sequence section, is selected.
8.4.3.3 Ensure **'Last'** is checked for the Calibration Tray in the control method section, so that the control will be measured after the measurement of the calibrator samples. Click **Save.**
8.4.3.4 Click **QC** from the main screen, and then select the **Control** button.
8.4.3.5 Enter the control sample name, e.g. TTxLow. Click **Save**.
8.4.3.6 Click **QC** from the main screen, and then select the **QC Range** button.
8.4.3.7 Double click on **TTxLow** from the item list. Enter the mean value and SD from the lot specific data sheet into the relevant boxes. The minimum and maximum allowed values for the control will automatically be entered. Press **Save** to update the changes and **Return** to exit.
8.4.3.8 Repeat for the high control.

8.4.4 Running calibration curves and controls

8.4.4.1 Request the calibration by clicking onto **Calib** from the Main screen and ensure the **CH ODR** box is checked for the desired assay.
8.4.4.2 Enter the position of the blank (sample diluent) and calibrators (standards) on the calibrator rack into the boxes, e.g. B1 – 1, S1 - 1, S2 – 1 etc. Ensure the calibrator numbers run consecutively.
8.4.4.3 The calibrator and controls are supplied ready for use. **Gently** mix by inversion just prior to use.
8.4.4.4 Fill a sample cup with sample diluent, and place in position B1 on the calibrator rack. Transfer 150µL of each calibrator into a sample cup and place them on the calibrator rack in ascending order (i.e. lowest concentration first), in the positions specified in step 8.4.4.2.
8.4.4.5 Click **Update** to save changes.
8.4.4.6 Controls can be assayed at the same time as the calibration using the same rack. Select **Order** from the main screen. Enter the control sample number in the Control no. box **(C1-C6).** Confirm the Order Status box shows 'Normal'. Select the required control from the drop down tab, and then select the tests the control is to be measured against. The standard sample dilution (1/10) will automatically be entered in the **Dil** box (10). As the concentrations of the controls are below the standard measuring range the dilution required must be changed by double clicking on the standard dilution figure (10) to display the redilution options and selecting the empty space above 10 to select neat measurements for both controls. Click on the empty space above 10 to select neat measurement for both controls.
8.4.4.7 To measure the controls, select **Control** from the main screen and click OK to accept automatic measurement of the control(s).
8.4.4.8 Ensure the controls are in the correct position on the cal rack (i.e. C1-C6).
8.4.3.1 If the controls are to be assayed from a sample rack, repeat the above but using the control positions for that rack, i.e. C7 – C12 on rack 1, C13 – C18 on rack 2 etc. Transfer 150µL of control into a sample cup and place on the rack.
8.4.4.9

8.4.5 Running patient samples

8.4.5.1 Place the sample on the sample rack in an empty position. Cups or primary tubes may be used.
8.4.5.2 From the Main Menu, click **Order.**
8.4.5.3 Click on **Position No.**, enter the sample carousel position number for the sample and press enter.
8.4.5.4 Click on **Patient ID** and enter an appropriate ID (name or sample number). Other patient information, e.g. Name, Sex, Age, DOB may be entered as desired according to each laboratories working practice.
8.4.5.5 Click on the assay **Name** to select the test.
8.4.5.6 The standard sample dilution (10) will automatically be entered in the **Dil** box.
8.4.5.7 Click on **Order** to complete the sample request.

8.4.6 Measuring range

Specificity	Approx. Measuring Range	
	IU/mL	Sample Dilution
T.Tox	1.56 – 50.0	1/10

9 QUALITY CONTROL

We recommend that the controls provided should be included in all assays performed. The anti-Tetanus toxoid immunoglobulin concentration is stated on the vial labels and QC Certificate. Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.

10 LIMITATIONS

10.1 These kits are not suitable for the measurement of samples containing rheumatoid factor, paraproteins, other circulating immune complexes (CIC's) or for lipaemic or haemolysed samples due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.

10.2 Customers are strongly advised to run both controls with every batch of samples being assayed. Should a control value be out of range against a stored curve, it is recommended that the assay be recalibrated. Where control values fall outside ±15% limit against new calibration curves check the instrument and parameters entered before repeating the assay. If problems persist, refer to the supplier.

11 EXPECTED VALUES

The result provided below has been obtained from a limited number of healthy adult UK blood donors and are intended for guidance purposes only. Wherever possible it is strongly recommended that local ranges are generated.

11.1 Adult Normal Ranges

120 normal adult donor sera were assayed for anti-Tetanus toxoid immunoglobulin on the SPA_{PLUS}. A range of <1.58 – 4.9 IU/mL was obtained. 68% of results were below the sensitivity limit of 1.58 IU/mL.

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A precision study was performed following NCCLS *Evaluation of Precision Performance of Clinical Chemistry Approved Guideline* (NCCLS Document EP5-A). The study was performed over 21 working days, with two runs per day. One user assessed three different samples using three different reagent lots on three analysers.

Tetanus toxoid Precision Summary									
	Mean (IU/mL))	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Plasma 1	2.9	0.02	0.8	0.06	2.0	0.13	4.6	0.15	5.0
Plasma 2	20.3	0.08	0.4	0.24	1.2	0.66	3.2	0.71	3.5
Plasma 3	44.1	0.26	0.6	0.5	1.1	2.06	4.7	2.13	4.8

12.2 Linearity

The linearity of this assay has been confirmed using diluted plasma samples over a range of 2.01 – 46.70 IU/mL, giving a regression equation of :

y=1.0x - 0.15 (IU/mL), R^2 *=0.9996.*

(y = measured anti-tetanus toxoid immunoglobulin concentration, x = theoretical concentration)

12.3 Interference

Minimal assay interference by 200mg/L bilirubin (+0.03%), 5g/L haemoglobin (+0.13%) and 8008.8 FTUs of Chyle (+1.46%) has been demonstrated using a sample containing 20.20 IU/mL of anti-Tetanus toxoid immunoglobulin, at the standard sample dilution (1/10).

12.4 Analytical Sensitivity

Analytical sensitivity was determined by assaying ten replicates of two samples with concentrations equivalent to 140% (2.10 IU/mL) and 200% (3.05 IU/mL) of the curve bottom point. Two distinct sets of data were generated with CV's of 1.92% and 1.50% respectively.

12.5 Comparison

A correlation study was performed on 486 plasma samples using this kit on a SPA_{PLUS} and on the Binding Site VaccZyme™ Tetanus toxoid IgG EIA kit (MK010.4). The study demonstrated a good agreement giving the following Passing Bablok plot and correlation coefficient:

y = 1.07x +0.47 IU/mL (y = SPA plus; x = EIA kit)

correlation coefficient R^2 = 0.87 (by linear regression)

VaccZyme™ is a trademark of The Binding Site Ltd., Birmingham, UK.

12.6 Antigen excess

The assay was tested to a level of >120IU/mL with a serum sample. No antigen excess was observed at this level. Samples believed to be above this level should be manually diluted before measurement (see section 8.4.5.6)

13 BIBLIOGRAPHY

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