Version 20-04/24

User's Manual

Human Anti-Diphtheria Toxin/Toxoid (DTX) IgA ELISA kit

REF **DEIAJX005**

Σ **96T**

RUO

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Human Anti-Diphtheria Toxin IgA ELISA Kit detects and quantifies diphtheria toxin (DTX) or toxoid-specific IgA in human serum or plasma of exposed or immunized hosts. This immunoassay is suitable for:

Determining immune status relative to non-immune controls;

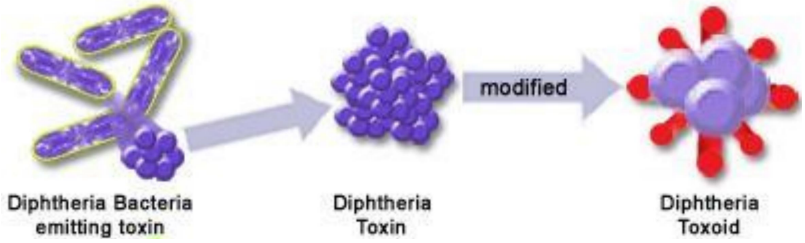
Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;

Qualifying and/or standardizing vaccine batches and protocols.

This kit is for research use only (RUO), not for diagnosis or therapeutic purposes.

General Description

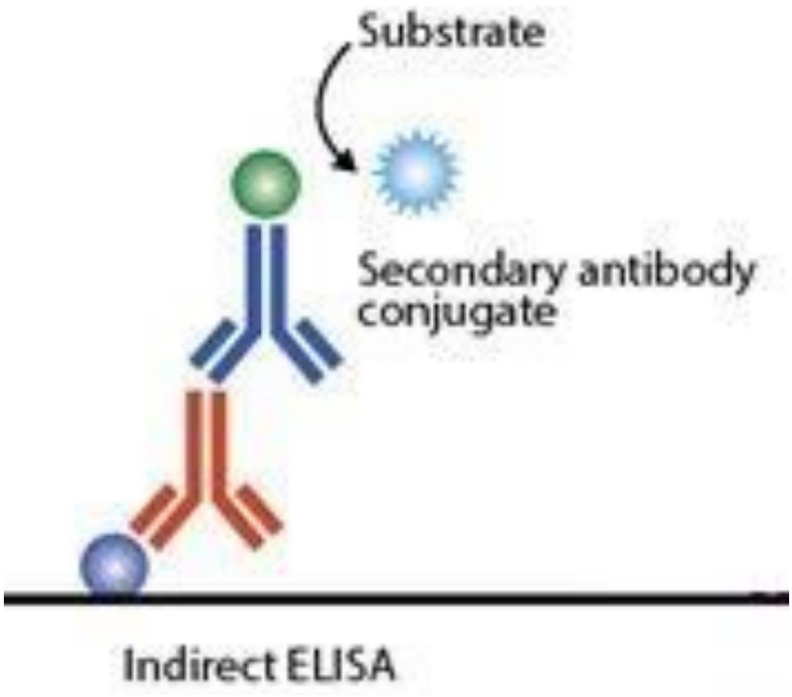
Diphtheria is a contagious disease of the upper respiratory tract. The illness is characterized by sore throat, low fever, and an adherent membrane (a pseudo membrane) on the tonsils, pharynx, and/or nasal cavity. Diphtheria also causes the progressive deterioration of myelin sheaths in the central and peripheral nervous system leading to degenerating motor control and loss of sensation. Common diphtheria has largely been eradicated in industrialized nations through widespread vaccination, e.g., the DPT (Diphtheria–Pertussis–Tetanus) vaccines are recommended for all school aged. These contain a toxoid of the disease-causing diphtheria toxin.



Monitoring the efficacy of vaccines by determining the anti-toxin levels in patients, including for clinical trials using new formulation of vaccines, is often required. The Creative Diagnostics Anti- Diphtheria ELISAs will quantify antibodies produced by vaccines, or from exposure to the toxin-producing organisms.

Principles of Testing

The Human Anti-Diphtheria Toxin/Toxoid (DTX) IgA ELISA kit is based on the binding of human anti-diphtheria toxin IgA in samples to diphtheria toxin immobilized on the microwells, and anti- diphtheria toxin IgA antibody is detected by anti- human IgA specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti- diphtheria toxin IgA present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of human IgA antibody in samples is calculated relative to anti- diphtheria toxin calibrators.



Reagents And Materials Provided

1. Wash Solution Concentrate (100x): 10ml
2. Sample Diluent Concentrate (20x): 10ml
3. Anti-Human IgAHRP Conjugate Concentrate (100x): 0.15ml
4. Diphtheria Toxin Coated Strip Plate: 8-well strips (12)
5. Anti- Diphtheria Calibrators: 10 U/ml 0.65ml, 25 U/ml 0.65ml, 50 U/ml 0.65ml, 100 U/ml 0.65ml
6. Anti- Diphtheria Positive Control: 0.65ml
7. Low NSB Sample Diluent: 30 ml
8. TMB Substrate: 12 ml
9. Stop Solution: 12 ml

Materials Required But Not Supplied

1. Pipettors and pipettes that deliver 100ul and 1-10ml.
2. Disposable glass or plastic 5-15ml tubes.
3. Stock bottle to store diluted Wash Solution; 0.2 to 1L.
4. Distilled or deionized water to dilute reagent concentrates.
5. ELISA reader at 450 nm and ELISA plate washer.

Storage

2-8°C until the expiration date printed on the box label.

Specimen Collection And Preparation

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Antibody Stability & Dilution

Initial dilution of serum into Working Sample Diluent (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into Low NSB Sample Diluent (LNSD), which provides the lowest assay background, should be at least 5 times the initial dilution and performed the same week as the assay.

Example: Initial (1/5): 10ul serum + 40ul WSD [or 0.1ml + 0.4ml]

Further (1/50): 10ul initial (1/5) + 90ul LNSD (1/50)

Plate Preparation

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample control to be assayed.

Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.

Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Reagent Preparation

1. Wash Solution Concentrate :Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at 4°C for long term and RT for short term.
2. Sample Diluent Concentrate :Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample/Conjugate Diluent and store at 2-8°C until the kit lot expires or is used up.
3. Anti-Human IgAHRP Conjugate Concentrate :Peroxidase conjugated anti-human IgA in buffer with detergents and antimicrobial. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample/Conjugate Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

Review Interpretation of Results before proceeding:

Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the 10 U/ml Calibrator. This is usually 1:200 or greater dilution for human serum with normal levels of IgA and IgM.

Run a Sample Diluent Blank. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.

Run the Anti-Diphtheria Positive Control; the value range is on the label

Run a set of Calibrators, which validate that the assay was performed to specifications: 100 U/ml should give a high signal (>1.5 OD); 10 U/ml should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results).

1. st Incubation [100ul – 60 min; 4 washes]

Add 100ul of calibrators, samples and controls each to predetermined wells.

Tap the plate gently to mix reagents and incubate for 60 minutes.

Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2 nd Incubation [100ul – 30 min; 5 washes]

Add 100ul of diluted Anti-Human IgA HRP to each well.

Incubate for 30 minutes.

Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.

Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

Add 100ul of Stop Solution to each well.

Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.

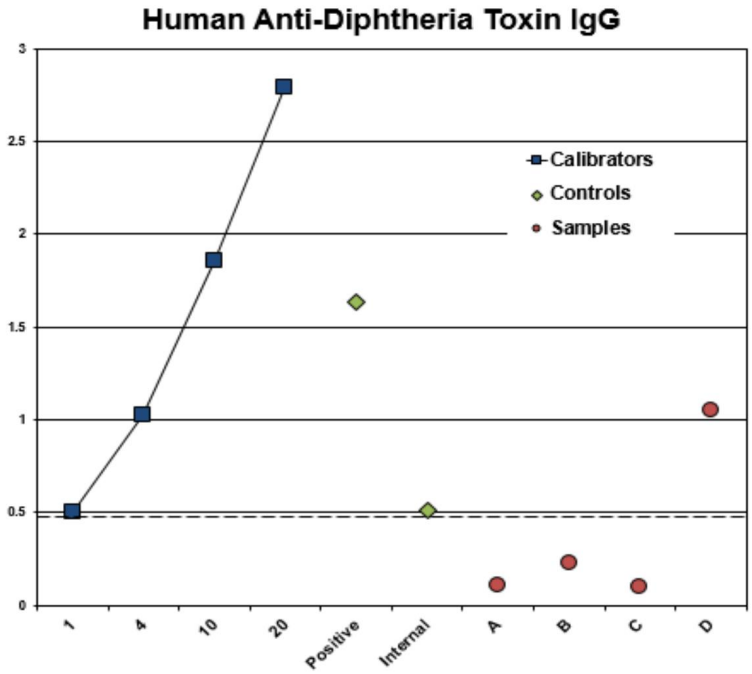
Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

Interpretation Of Results

Method A. Antibody Activity Threshold Index

Compare Samples to 10 U/ml Calibrator or Internal Control = Positive/Negative Cut-off.

Example:



Results

The sensitivity of the assay to detect anti-diphtheria toxin IgA, from either natural infection or vaccination, is controlled so that the 10 U/ml Calibrator represents a threshold OD for most true positives in human serum diluted to 1:200 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of antiserum from diphtheria toxin immunization, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.

10 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between Positive/Negative. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – antiserum from vaccination with diphtheria toxin; value range is on the vial label. This Control can be used to assess reproducibility and to normalize between-assay variation.

Internal Control – a true positive from an immune human that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the 10 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (1:100) (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

The 10 U/ml Calibrator can be used to calculate a Threshold Index that numerically discriminates Positive/Negative:

Divide each Sample net OD by the 10 U/ml Calibrator net OD. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody.

Method B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a Positive Index. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Nonimmune samples = Positive Index.

2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 is Negative for antibody.

A sample value would be Positive if significantly above the value of the pre-immune serum sample or a suitably determined nonimmune panel or pool of samples, tested at the same sample dilution.

This calculation also quantifies the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Example:

	Assay Net OD		Calculated Antibody Activity	
Sample	Control	Exptl	Control	Exptl
1	0.244	**C** 2.293	0.57	**5.34**
2	0.204	**C** 1.490	0.48	**3.47**
3	0.237	**C** 0.833	0.55	**1.94**
4	0.26	**C** 0.326	0.61	0.76
5	0.388	**P** 1.106	0.90	**2.58**
6	0.407	**I** 0.310	0.95	0.72
7	0.288	**E** 0.672	0.67	**1.56**
8	0.263	**E** 0.363	0.61	0.85
9	0.322	**E** 0.560	0.75	**1.31**
10	0.343	**E** 0.490	0.80	1.14
Mean	0.295			
SD	0.067			
Mean +2 SD	**0.429**	= **Positive Index**		

Results

Experimental Samples are represented as follows:

C – Calibrator

P – Positive Control

I – Internal Control; lab's threshold positive serum

E – Experimental sample

Method C. Titers from Sample Dilution Curves

A sample value would be Positive if significantly above the value of the pre-immune serum sample or a suitably determined nonimmune panel or pool of samples, tested at the same sample dilution.

This calculation also quantifies the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

The titer of elevated antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. The Calibrator values can be used to normalize inter-assay values.

Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index

= IgA Antibody Activity Units

Calibrator Curve Quantitation

antibody activity from a calibrator curve (such as provided with To quantitate the kit), the dilution curve of the samples must be parallel to the calibrator curve, to avoid different values being obtained from different regions of the curve. In cases of nonparallelism, antibody activity is best expressed as a titer relative to the titer of a reference positive, as shown above.

Sensitivity

The diphtheria toxin coating level, HRP conjugate concentration and Low NSB Sample Diluent are optimized to differentiate anti- diphtheria toxin/toxoid IgA from background (non-antibody) signal with human serum samples diluted 1:200.

Specificity

N/A

Precautions

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.