



**User's Manual**

# **Monkey Anti-Diphtheria Toxin/Toxoid (CRM197) IgG ELISA kit**

**REF**

**DEIAJX003**



**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.

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**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](mailto:info@creative-diagnostics.com)**  **Web: [www.creative-diagnostics.com](http://www.creative-diagnostics.com)**

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## PRODUCT INFORMATION

### Intended Use

This kit is for measuring anti-CRM197 IgG in monkey serum or plasma samples. For in vitro research use only.

### General Description

Diphtheria (Greek diphthera)—"pair of leather scrolls" is an upper respiratory tract illness characterized by sore throat, low fever, and an adherent membrane (a pseudo membrane) on the tonsils, pharynx, and/or nasal cavity. A milder form of diphtheria can be restricted to the skin. It is caused by *Corynebacterium diphtheriae*, an aerobic Gram-positive bacterium. Diphtheria causes the progressive deterioration of myelin sheaths in the central and peripheral nervous system leading to degenerating motor control and loss of sensation. Diphtheria is a contagious disease spread by direct physical contact or breathing the aerosolized secretions of infected individuals. In the 1920s there were an estimated 100,000 to 200,000 cases of diphtheria per year in the USA, causing 13,000 to 15,000 deaths per year. Children represented a large majority of these cases and fatalities. Common diphtheria has largely been eradicated in industrialized nations through widespread vaccination. DPT (Diphtheria–Pertussis– Tetanus) vaccine is recommended for all school aged children. Boosters of the vaccine are recommended for adults since the benefits of the vaccine decrease with age without constant re-exposure; they are particularly recommended for those traveling to areas where the disease has not been eradicated. Diphtheria toxin consists of a single polypeptide. Proteolysis yields two fragments (A and B) which are held together by a disulfide bond. The toxin binds to EGF-like domain of Heparin-binding EGF-like growth factor (HB-EGF) through fragment B and is internalized with HB-EGF by receptor-mediated endocytosis. The low pH in the late endosomes induce pore formation by fragment B as well as catalyses the release of catalytic fragment A into the cytosol. Diphtheria toxin catalyzes the ADP-ribosylation of, and inactivates, the elongation factor eEF-2. In this way, it acts to inhibit translation during eukaryotic protein synthesis. The toxin enters the host cell and is hydrolysed by a trypsin-like protease to give a fragment with enzymatic activity. The toxin then transfers an ADP-ribose from NAD<sup>+</sup> to a diphthamide residue, a modified histidine (amino acid), which is found within the EF-2 protein. EF-2 is needed for translocation of tRNA from the A-site to the P-site of the ribosome during translation. The ADP-ribosylation is reversible by administering high concentrations of nicotinamide, one of the reaction products.

There are several Diphtheria vaccines available that can be used alone or in combination with other diseases (multivalent). It is often necessary to monitor the efficacy of vaccines and determine the anti-Diphtheria Ig levels in patients or for clinical trial using new formulation of vaccines. Monkey Anti- CRM197 IgG ELISA kit is an immunoassay for the quantitative determination of IgG class antibodies against CRM197 in monkey serum and plasma. Diphtheria Vaccines: Pediarix (DTAP/HepB/IPV), Infanrix (DTAP), Boostrix (Tetanus, Diphtheria, Acellular Pertussis) – GlaxoSmithKline; Trihibit (DTAP/Hib), Daptacel (DTAP), Tripedia (DTAP), DT (Pediatric), Td (Adult), Decavac<sup>TM</sup> (tetanus/Diphtheria), Adacel (tetanus, Diphtheria, Acellular Pertussis) Sanofi Pasteur.

### Reagents And Materials Provided

1. CRM197 antigen pre-coated, stabilized, ready-to-use 96-well strip plate, suitable for multiple runs over 6-12 months.

2. Convenient, stable, liquid standards: 0, 0.01, 0.10, 0.50, 1 IU/mL containing monkey anti- CRM197 IgG in a stabilizing buffer.
3. Sample Diluent.
4. Antibody-HRP Conjugate.
5. TMB Substrate solution.

## Storage

12 months.

## Assay Procedure

Step 1. Pipet 100 ul each of pre-diluted standards, samples (diluted 1:101 or more). Mix gently and incubate at room temp for 60 min.

Step 2. Aspirate and wash 3X. Add 100 ul of antibody-HRP Conjugate to all wells, mix gently and incubate at room temp for 30 min.

Step 3. Aspirate and wash 4X. Add 100 ul of TMB Substrate solution to all wells, mix gently, and incubate at room temp for 20 min.

Step 4. Pipet 100 ul of stop solution into each well and mix gently (blue color turns yellow). Measure absorbance at 450 nm. Determine antibody concn in each sample using the calibrators (results are expressed as positive or negatives or in units/ml).

## Interpretation Of Results

< 0.1 IU/mL: Basic immunization recommended 0.1 - 1.0 IU/ml booster vaccination recommended 1.0 - 1.5 IU/ml to be boosted in 5 yrs 1.5 - 2.0 IU/ml to be boosted in 7 yrs > 2.0 IU/ml to be boosted in 10 years

## Precision

Intra-Assay-Precision: 4.9 %

Inter-Assay-Precision 3-7%

## Sensitivity

0.004 IU/mL

## Specificity

No significant cross-reactivities known.

No cross-reactivity to Clostridium tetani.

## Linearity

78–133%

## Recovery

96–102 %

## Interferences

No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL und triglycerides up to 5.0 mg/mL.