

## DRG<sup>®</sup> IgA CIC ELISA (EIA-3988)



Revised 22 Nov. 2010 rm (Vers. 1.1)

**RUO** in the USA

### 1. INTENDED USE

Immunoenzymatic colorimetric method for determination of IgA-CIC concentration in serum and plasma.

**This kit is intended for Research Use Only. Not for use in diagnostic procedures.**

### 2. PRINCIPLE

C3-fixing circulating immune complexes (CIC) are first blocked by an anti-C3 antibody immobilized on a microplate. To quantitate IgA-CIC complexes a specific peroxidase-conjugated anti-IgA antibody and H<sub>2</sub>O<sub>2</sub>-TMB as the enzyme substrate are then employed.

Amounts of the enzyme reaction product formed, which are measured by reading absorbance at wavelength 450 nm, are proportional to the levels of solid-phase IgA-CIC complexes.

### 3. REAGENTS, MATERIALS AND INSTRUMENTATION

#### 4. Reagents and materials supplied with kit

(sufficient for 96 wells-determinations)

1. **Incubation buffer** (100 mM Borate buffer, pH 7.4, 10 g/L Bovine Serum Albumin); 27 mL
2. **Conjugate** (peroxidase-conjugate anti-IgA); 0.75 mL
3. **Conjugate buffer** (100 mM Borate buffer, pH 7.4, 10 g/L Bovine serum albumin); 25 mL
4. **Substrate** (H<sub>2</sub>O<sub>2</sub>-TMB 0.25 g/L); 12 mL
5. **Stop solution** (H<sub>2</sub>SO<sub>4</sub> 0.15M ); 12 mL
6. **Microassay plate** (strips coated with Anti-C3 (Fab')<sub>2</sub>).

#### 5. Note

- All reagents and the microplate should be stored at 2-8°C in the dark and used within the date written on the package.
- Leave the microplate at room temperature for few minutes prior to removing a number of wells necessary for the assay.
- Place the unused microwell strips into the storage bag and reseal the bag with tape.

#### 6. Reagent required but not supplied with kit

0.9% NaCl solution

#### 7. Materials

Glass or plastic test tubes (approximately 5 ml);

Vortex

Micropipettes, adjustable multichannel pipette or repeating micropipettes;

37°C incubator;

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Microplate reader capable of reading A<sub>450</sub> values.

### 8. Preparation of reagents

#### Dilution of the Conjugate

For each series of 16 wells, dilute 100 µl of Conjugate (reagent 2) in 2 ml of Conjugate buffer (reagent 3).

Mix well and avoid foaming.

Reagents are stable three hours at room temperature.

### 9. SAMPLE PREPARATION

The CIC assay can be performed in both serum and plasma.

Samples which are not immediately processed should be stored at -20°C.

Samples should not be thawed more than once.

### 10. CONTROLS

It should be mentioned that a suitable parameter for comparing concentration of CIC is not currently available.

Generally, for comparison purposes, it is considered the concentration of IgG complexes which, as CIC, can combine with the complement.

However, there are several limitations in using IgG complexes to estimate levels of CIC:

- only CIC containing IgG can be measured (the procedure cannot be adapted to IgA, IgM and IgE-containing CIC);
- IgG complexes are not stable;
- products of temperature aggregation may not be reproducible.

In view of these observations, it has been suggested that a more suitable control is represented by a population of healthy blood donors.

**A screen of 50-80 individuals is sufficient to establish a "normal range" of values.**

**Assay-to-assay variations may be eliminated by performing each time duplicate determination of 6-10 control samples.**

### 11. Precautions

- Sera of control individuals may be stored at -20°C for several months.
- Samples should be dispensed in small aliquots (50 µl) and thawed only once.
- Do not use either lyophilized serum or pool of sera as controls.
- Handle control and sample sera in the same way.

### 12. PROCEDURE

#### 13. Sample/Control dilution.

Pipette in a test tube:

Serum/plasma 25 µl

Incubation Buffer (reagent 1) 500 µl

Vortex.

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## 14. Assay Procedure.

As it is necessary to perform in duplicate, each determination should also include two wells for each Sample, two wells for any Control and two wells for Blank.

|                               | Sample/Control | Blank  |
|-------------------------------|----------------|--------|
| Diluted Serum/plasma          | 100 µl         | ---    |
| Incubation buffer (reagent 1) | ---            | 100 µl |

Incubate at 37°C for 30 minutes.

Remove the contents from each well.

Wash three times each well with:

|                             |        |        |
|-----------------------------|--------|--------|
| Saline solution (0.9% NaCl) | 300 µl | 300 µl |
|-----------------------------|--------|--------|

Leave the excess liquid to drain away by inverting the plate on absorbent paper (2-3 min.).

Dispense into each well:

|                   |        |        |
|-------------------|--------|--------|
| Diluted conjugate | 100 µl | 100 µl |
|-------------------|--------|--------|

Incubate at 37°C for 30 minutes.

Remove the contents from each well.

Wash three times each well with:

|                             |        |        |
|-----------------------------|--------|--------|
| Saline solution (0.9% NaCl) | 300 µl | 300 µl |
|-----------------------------|--------|--------|

Leave the excess liquid to drain away by inverting the plate on absorbent paper (2-3 min.).

Dispense into each well:

|               |        |        |
|---------------|--------|--------|
| TMB-Substrate | 100 µl | 100 µl |
|---------------|--------|--------|

Incubate at room temperature for 15 min

Dispense:

|               |        |        |
|---------------|--------|--------|
| Stop solution | 100 µl | 100 µl |
|---------------|--------|--------|

Determine the absorbance (E) by reading at 450 nm for each test-well (within 30 minutes after the addition of the Stop solution) after zeroing the instrument with the Blank.

## 15. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

## 16. BIBLIOGRAPHY

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