DRG® CH50 ELISA (EIA-3989)

CE

REVISED 29 SEPT. 2010 RM (VERS. 10.1) **RUO IN THE USA**

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

Immunoenzymatic colorimetric method for determination of complement functionality in human serum.

2 PRINCIPLE

The complex β-galactosidase/anti-β-galactosidase, is solubilized by serum through the deposition of C3b molecules. The formation of C3b quantity necessary for the solubilization is mediated by alternative pathway, but it is accelerated from activity of C3-convertase by classic way.
The quantity of complex β-galactosidase dissociated, detectable by enzymatic activity in the supernatant at the end of reaction; represents the capacity of serum to form C3b molecules.
The o-nitrophenil-galactopiranoside (o-NPG) is used as substrate and the reagent product (o-nitrophenol) is read at 420nm (or 405 nm).

3 REAGENTS, MATERIALS AND INSTRUMENTATION

3.1 Reagents and materials supplied in the kit

1. **Reference Calibrator** (1 vial) 0.6 mL
   Ready to use
2. **Incubation Buffer** (1 vial) 12 mL
   Phosphate buffer 50 mM pH 7.35;
3. **Immunocomplex** (1 vial) 6 mL
   □□□□□□β-galactosidase/anti-β-galactosidase,
4. **Microplate,** 1x, breakable
5. **oNPG-Substrate** (1 vial, lyophilzed) (see 4.2 "Preparation")
   Phosphate buffer 15 mM, pH 7.0; o-NPG 2.3 mM - (avoid any skin contact)
6. **Ethandiol** (1 vial), 1.0 mL
7. **Stop Solution** (1 vial) (7 mL)
   Sodium Carbonate 16%, - (avoid any skin contact).
8. **Control** with different levels of solubilisation, (0.6 mL)
   Low Control (1 vial)
   High Control (1 vial)

3.2 Reagents necessary not supplied

Distilled water.

3.3 Auxiliary materials and instrumentation

Automatic dispenser

Microplates reader (filter at 420 or 405 nm)
Incubator 37°C
Centrifuge (10000 - 13500 x g "RCF") (RCF = Relative Centrifugal Force)
Reference Serum (1 vial) Lyophilised

3.4 Note

The Reference Calibrator and Controls are synthetic; they guarantee higher reproducibility and stability compared with the reference of human origin.
Store all reagents at 2 °C – 8 °C in the dark.
Bring all reagents to room temperature 22 °C – 28 °C.
Maintain the same order in reagent dispensation.
Use only serum samples (avoid using plasma samples)
Human serum is stable for one month at –20°C (six months stored frozen at -80 °C).

4 PROCEDURE

4.1 Preparation of the Immune Complex

Use the reagent without any dilution.
Before use mix well the immunocomplex with vortex.
Stable for 3 months at 2 °C – 8 °C

4.2 Preparation of the ONPG-Substrate

Add 10 mL of distilled water to the reagent. Once the reagent is dissolved, add 0.5 mL of Ethandiol.
Stable for 2 months at 2 °C – 8 °C.

IMPORTANT!
For a better repeatability (inter-assay), we suggest to bring the substrate at room temperature (22-28°C) before use (avoid the dispensation of reagent just removed from the fridge)

4.3 PROCEDURE

Step 1 in Eppendorf tubes

Dispense each serum sample, the reference calibrator and control and a not solubilising control in an Eppendorf tube:

	Reference Calibrator	Sample/ Control	Not-solubilising Control
Incubation Buffer	100 µL	100 µL	150 µL
Reference Calibrator	50 µL	---	---
Sample	---	50 µL	---
Immunocomplex	50 µL	50 µL	50 µL

Mix well and incubate *2 hours* at 37°C.
Centrifuge at 10000-13500 xg "RCF" for 15 minutes.
Transfer with care, avoid touching the pellet with the pipette, 50 µL of supernatant of each Eppendorf tube in the well of microplate.

IMPORTANT!

- *Avoid the suspension of the pellets.*
- *Do not shake the centrifugate.*
- *Take slowly the supernatant in order to avoid turbulences that cause the suspension of pellet*

[The pellet is composed of not solubilised immuncomplex with high enzymatic activity (β-Galctosidase), the presence of a small quantity of pellet in the supernatant can cause false positives and erroneous values for controls

Step 2 in the Microplate

	Blank	Reference Calibrator	Sample/ Control	Not-solubilising Control
Incubation Buffer	50 µL			
Supernatant		50 µL	50 µL	50 µL
oNPG Substrate) (room temperature)	100 µL	100 µL	100 µL	100 µL
Incubate for 15 minutes at +37°C in the dark.				
Stop Solution	50 µL	50 µL	50 µL	50 µL
Shake the microplate gently. Read the absorbance (O.D.) at 420 nm (405 nm) against the Blank.				

5 QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of CH50 for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends.
The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

6 RESULTS

6.1 Mean absorbance

Calculate the mean of the absorbencies (OD) of reference calibrator, control and of each sample.

6.2 Calculation of results

The result can be expressed as
a. CH50 value or as
b. % of Reference Calibrator

The exact CH50 Value of Reference Calibrator is lot-dependent and is reported on the label.

Determinate the results using the following formula:
a. $OD_{Sample} / OD_{Reference\ Calibrator}$ x CH50 (Value of Reference Calibrator) = CH50 Value of sample

b. $OD_{Sample} / OD_{Reference\ Calibrator}$ x CH50 (% of Reference Calibrator) = % of Reference Calibrator

Example:
CH50 Value of Reference Calibrator Vial = 100
CH50 % of Reference Calibrator Vial = 50

Absorbance of Reference Calibrator = 0.350
Absorbance of Sample = 1.108

a. CH50 Value of Sample = 1.108/0.350 × 100 = 316
b. % of Reference Calibrator = 1.108/0.350 × 50 = 158 %

7 BIBLIOGRAPHY

1. Miller G.W, Nussenzweig V.: A new complement function: solubilization of antigen-antibody aggregates. PNAS, 72, 418 – 1975.
2. Takahaschi M., Takahaschi S., Brade U., Nussenzweig V.: Requirement for the solubilization of immuno-aggregates by complement. J. Clin. Invest. 62, 349 – 1978.
3. Migliorini P, et al. J. of Immunological Methods, 77 119-130 (1985)