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# ZYMUTEST HIA IgA

**Ref RK040C-RUO (96 tests)**

Qualitative assay for the detection of heparin-dependent antibodies of the IgA isotype by ELISA

**FOR RESEARCH USE ONLY.**

**NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

English, last revision: 04-2018

## INTENDED USE:

The ZYMUTEST HIA, IgA kit, is an optimized ELISA test intended for the detection and the qualitative assay of heparin-dependent antibodies of the IgA isotype, in human plasma or serum or any other biological fluids where these antibodies must be detected.

**This kit is for research use only and should not be used for patient diagnosis or treatment.**

## SUMMARY AND EXPLANATION:

This assay is designed with biologically available and immobilized heparin, then stabilized and saturated, which allows reacting fully with heparin binding proteins and antibodies. This reliable method then provides high reproducibility, high sensitivity and high specificity by identifying IgA isotype heparin-dependent antibodies, and by mimicking the binding mechanism of antibodies in vivo, on heparin present at the cell surface, especially on platelets or endothelial cells.

## ASSAY PRINCIPLE:

The diluted assayed plasma or serum sample is introduced into one of the microwells of the coated plate, and supplemented with a platelet lysate. When present, heparin-dependent antibodies, of the IgA isotype, form complexes onto the biologically available unfractionated heparin, immobilised and saturated. Following a washing step, bound antibodies are revealed with the immunoconjugate, which is made of goat polyclonal antibodies anti-human IgA (α specific)-peroxidase (HRP) conjugate. This immunoconjugate reacts specifically with IgA isotypes. Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of hydrogen peroxide ($H_2O_2$), is introduced and a blue colour develops. The colour turns yellow when the reaction is stopped with sulfuric acid. The colour developed is directly proportional to the amount of heparin-dependent antibodies, of the IgA isotype, present in the tested sample.

## REAGENTS:

1. **COAT: Micro ELISA plate,** containing 12 strips of 8 wells, coated with unfractionated heparin, biologically available, saturated, then stabilized; the plate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
2. **SD:** 2 vials containing 50 mL of **HIA Sample Diluent**, ready to use. Contains Sodium Azide
3. **C+:** 3 vials of **HIA IgA Positive control**, lyophilised. When restored with **1 mL** of **HIA Sample Diluent**, the ready to use positive control is obtained (already diluted 1:100). The expected reactivity ($OD_{450nm}$) is indicated on the flyer provided with the kit.
4. **C-:** 3 vials of **negative control**, lyophilised (diluted normal human plasma). When restored with **1 mL** of **HIA Sample Diluent**, the ready to use negative control is obtained (already diluted 1:100). Contains BSA.
5. **CLy:** 3 vials of **cell lysate** lyophilised containing diluted normal human plasma. When restored with **2 mL** of **distilled water**, the ready to use solution is obtained. Contains BSA.
6. **IC:** 3 vials of **immunoconjugate (Anti-IgA (α)-HRP immunoconjugate)**, goat polyclonal antibodies specific to the α coupled to HRP of human IgA, and lyophilised. When restored with 7.5 mL of Conjugate Diluent (**CD**), the ready to use immunoconjugate is obtained. Contains BSA.
7. **CD:** 1 vial of 25 mL of **conjugate diluent**, ready to use. Contains BSA.
8. **WS:** 1 vial of 50 mL of **Wash Solution**, 20 fold concentrated.
9. **TMB:** 1 vial of 25 mL peroxidase substrate: **3,3',5,5' – Tetramethylbenzidine** containing hydrogen peroxide, ready to use.
10. **SA:** 1 vial of 6 mL of **0.45M Sulfuric Acid (Stop Solution)**, ready to use.

Reagent SD contains low concentration of Sodium azide (0.9 g/L) and reagent SA contains sulfuric acid, see CAUTIONS AND WARNINGS

## CAUTIONS AND WARNINGS:

- Any product of biological origin must then be handled carefully, as being potentially infectious.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.
- If the TMB substrate becomes yellow, this indicates the presence of a contaminant. It must be rejected, and a new vial must be used.
- The disposal of waste materials must be carried out according to current local regulations
- Use only reagents from kits with the same lot number. Do not mix reagents from kits with different lots when running the assay; they are optimized for each lot of kits.
- Reagents must be handled with care, in order to avoid any contamination during use. Take care to limit as much as possible any evaporation of the reagents during use, by limiting the liquid-air surface exchange.
- In order to preserve the stability of the reagents, close the vials with their original screw cap following each use.
- Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.
- The bovine plasma used to prepare the BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.
- Sulfuric acid, although diluted to 0.45M is caustic. As for any similar chemical, handle Sulfuric acid with great care. Wear protection glasses and gloves when handling. Avoid any skin and eye contact.
- For *in vitro* use.

**SA :** H290: May be corrosive to metals.
**CLy:** H315: Causes skin irritation.
H319: Causes serious eye irritation.
**CD, WS :** H317: May cause an allergic skin reaction.

## PREPARATION AND STABILITY OF REAGENTS:

Bring the kit at room temperature, at least 30 min before use. Store the unused reagents at 2-8°C. Vials are closed under vacuum. Remove carefully the stopper of lyophilized reagents, in order to avoid any loss of powder when opening the vials.

When appropriately used and stored, according to the recommended protocol and cautions, the kit can be used over a two month period, and strip by strip, if required.

1. **COAT (Micro ELISA plate):** Open the aluminium pouch and take off the required amounts of 8 well strips for the test series. When out of the pouch, the strips must be used within 30 minutes. Unused strips can be stored at **2-8°C for 8 weeks** in their original aluminium pouch, in presence of the desiccant, hermetically closed and protected from any moisture, and stored in the provided plastic microplate storage bag (minigrip).
2. **SD (HIA Sample Diluent):** Ready to use. This reagent contains sodium azide. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial is:
   - **8 weeks** at 2-8°C
3. **C+ (HIA IgA Positive Control):** Reconstitute each vial with **1 mL** of **"HIA Sample Diluent"**, shake thoroughly for complete dissolution. The obtained control is ready to use and it corresponds to a plasma containing IgA isotype heparin dependant antibodies, already **diluted 1:100**.
   Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial is:
   - **2 weeks** at 2-8°C.
   - **2 months** frozen at -20°C or below.
4. **C- (Negative Control):** Reconstitute each vial with **1 mL** of **"HIA Sample Diluent"**, shake thoroughly for complete dissolution. The obtained negative control is ready to use and it corresponds to a normal human plasma, already **diluted 1:100**.
   Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **2 weeks** at 2-8°C.
   - **2 months** frozen at -20°C or below.
5. **CLy (Cell lysate):** Reconstitute each vial with **2 mL** of distilled water, shake thoroughly for complete dissolution. The obtained reagent is ready to use.
   Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial is:
   - **2 weeks** at 2-8°C
   - **2 months** frozen at -20°C or below.
6. **IC (Anti-IgA(α)-HRP immunoconjugate):** Reconstitute each vial with **7.5 mL of Conjugate Diluent** at least 15 min before use. Let the pellet to be completely dissolved before use, and shake the vial gently in order to homogenize the content.
   Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **4 weeks** at 2-8°C.
   - **24 hours** at room temperature (18-25°C).
   - **2 months** frozen at -20°C or below.
7. **CD (Conjugate Diluent):** Ready to use. This reagent contains 0.05% Kathon CG. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **8 weeks** at 2-8°C
8. **WS (Wash Solution):** Incubate, if necessary, the vial in a water bath, at **37°C**, until complete dissolution of crystals. Shake the vial and dilute the amount required **1:20** in distilled water (the 50 mL contained in the vial allow to prepare 1 liter of Wash Solution).
   Stability of the wash solution, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **8 weeks** at 2-8°C

   Stability of the dilute wash solution, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **When open, 7 days** at 2-8°C

   This reagent contains 0.05% Kathon CG.
9. **TMB:** Ready to use. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **8 weeks** at 2-8°C
10. **SA (Stop Solution):** Stop Solution contains 0,45M sulfuric acid, ready to use. Stability reagent after opening, provided that any contamination or evaporation is avoided, kept in its original vial is:
    - **8 weeks** at 2-8°C

## STORAGE CONDITIONS:

Unopened reagents must be stored at 2-8°C, in their original packaging box. They are usable until the expiration date printed on the kit.

## REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

***Reagents:***

- Distilled water.

***Materials:***

- **8-channel** or **repeating pipette** allowing dispensing volumes of 50-300 µL.
- **1-channel pipettes** at variable volumes from 0 to 20 µL, 20 to 200 µL and 200 to 1000 µL.
- **Micro ELISA plate washing equipment** and **shaker**.
- **Micro ELISA plate reader** with a wavelength set up at 450 nm.

## SPECIMEN COLLECTION AND PREPARATION:

Preparation and storage of specimens must be performed according to the current local regulations.

- Specimens:

Human plasma obtained from trisodium citrate anticoagulated blood. EDTA collected human plasma may also be used. The storage conditions are the same with citrated plasma. Heparin dependent antibodies can also be assayed on serum. The serum is then prepared in the usual manner for the laboratory assays. Decant serum from clot before using or freezing. The storage conditions are identical to those of plasma.

- Collection:

Blood (9 vol.) must be collected on trisodium citrate anticoagulant (1 vol.) (0.109M), with caution, through a net venipuncture. The first tube must be discarded.

- Centrifugation:

Within 2 hours, use a validated method in the laboratory to obtain a platelet-poor plasma, e.g., a minimum of 15 minutes at 2500 g at room temperature (18-25°C) and plasma must be decanted into a plastic tube.

- Storage of plasma:
  - 24 hours at room temperature (18-25°C).
  - 6 months at -20°C.

Frozen plasma specimens should be rapidly thawed at 37°C, then gently mixed and tested within 72 hours. Resuspend any precipitation by thorough mixing immediately after thawing and before testing.

## TEST PROCEDURE:

**Assay procedure:**

1. Controls are ready to use (**already diluted 1:100**).

2. The samples should be diluted using SD solution as described in the table below:

| Samples | Dilution |
|---|---|
| Plasma | 1:100 |
| Serum | 1:100 |
| Biological fluid | 1:100 |

When high amounts of heparin dependent antibodies are expected, dilute at **1:200** or **1:400** dilutions. Results must then be multiplied by **2** or **4**.

3. Remove the required number of strips from the aluminium pouch and put the strips in the frame provided. In the different wells of the micro ELISA plate, introduce the reagents and perform the various assay steps as indicated on the following table:

| Reagent | Volume | Procedure |
|---|---|---|
| **CLy** | **50µL** | Introduce the CLy into the micro ELISA plate wells |
| **IgA Positive control** or **Negative control** or **1:100 diluted sample** or **sample diluent** (blank) | **200 µL** | Introduce immediately the dilutions :<br>– **IgA Positive control** or<br>– **Negative control** or<br>– **Diluted sample** or<br>– **Sample diluent**<br>into the micro ELISA plate wells **(a)** |
| **Incubate for 60 minutes at room temperature (18-25°C) (b)** | | |
| **Wash Solution** (20 fold diluted in distilled water) | **300 µL** | Proceed to 5 successive washings **(c).** |
| **Immunconjugate** (anti-IgA (α)-HRP immunoconjugate, reconstituted with 7.5 ml of conjugate diluent) | **200 µL** | Immediately after the washing, introduce the **immunoconjugate** in the micro ELISA plate wells**(c)** |
| **Incubate for 60 minutes at room temperature (18-25 °C) (b)** | | |
| **Wash Solution** (20 fold diluted in distilled water) | **300 µL** | Proceed to 5 successive washings **(c)**. |
| **TMB/$H_2O_2$ Substrate** | **200 µL** | Immediately after the washing, introduce the substrate into the wells.<br>**Nota**: The substrate distribution, row by row, must be accurate **(c,d)** |
| Let the colour develop for exactly **5 min**. at room temperature (18-25 °C) **(b)** | | |
| **0.45M Sulfuric Acid (Stop Solution)** | **50 µL** | Following exactly the same time intervals than for the addition of substrate, stop the colour development by introducing the **0.45M** Sulfuric Acid **(c,d)** |
| **Wait for 10 minutes in order to allow the colour to stabilize and measure absorbance at 450nm. Substract the blank value (e).** | | |

**Remarks:**

**a)** Distribute controls and tested specimen as rapidly as possible (within 10 minutes), in order to obtain an homogeneous immunological kinetics for antibodies binding. A too long delay between the distribution of the first and the last wells may induce an influence of immunological kinetics and produce wrong results.

**b)** Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro-ELISA plate shaker can be used. An incubation temperature of 18-25°C must be respected. Results are affected by a too high (>25°C) or too low (<18°C) temperature, and measured A450 are then too high or too low. It has to be considered when analyzing the results. In the same way, if a microplate shaker is used, it should be used only at the beginning of each step (sample introduction, immunoconjugate introduction, stop solution introduction), for 1 to 2 minutes, in order to obtain a good homogeneity. A450 values generated in the assay are significantly increased if shaking is used throughout the incubation steps.

**c)** Never let the plates empty between the addition of the reagents or following the washing step. The next reagent must be added within 3 minutes, in order to prevent the plate from drying, which could damage the immobilized components. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument must be adjusted in order to wash the plates gently, and to avoid a too drastic emptying, which could lower plate reactivity.

**d)** For addition of the TMB substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction.

**e)** For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used.

## VALIDATION:

- Controls provided in the kit allow validating the right performance of the assay.
- Expected OD values for positive control and the negative control can present variations from lot to lot but, when the assay is performed at room temperature, between 18 and 25°C, they always are:

**P =$OD_{450}$ for C+ 1:1: ≥ 1.0** **N = $OD_{450}$ for negative control: ≤ 0.25**

Obtained values for P and N, at 20±1°C, are indicated on the flyer provided in the kit.
Obtained A450 can vary according to the effective temperature during the assay run.

## RESULTS:

- Results are expressed according to the A450 values, as positive or negative.
- When higher dilutions are used, the complementary dilution factor must be considered.

**The results obtained should be for research purposes only and not used for patient diagnosis or treatment.**

## LIMITATIONS:

- In order to get the optimal performances of the assay, the technical instructions must be strictly respected.
- Any reagent presenting an unusual aspect or contamination signs must be rejected.
- Any plasma containing contamination signs must be rejected.
- If washing steps are not correctly performed, it can induce high background and a high absorbance value of the negative control. In order to avoid non-specific colour development, check that the washing step is efficiently and correctly performed.

## CHARACTERIZATION OF POSITIVE SAMPLES (IF REQUIRED):

If required, positive samples can be further characterized by their binding inhibition in presence of heparin. For this confirmation, to 500 µL of the 1:100 diluted tested specimen (plasma or serum), add 10 µL of a 100 IU/mL Unfractionated heparin solution and mix homogeneously. This heparinized solution (2 IU/mL final) must then be tested in the assay. Heparin dependent antibody binding to the plate is then inhibited (decrease in absorbance more than 50%) in almost all the cases. This inhibition confirms the heparin dependent binding of antibodies.

## PERFORMANCES:

- No interference of Heparin up to 1 IU/mL.
- Inter assay: <10%.
- Intra assay: <10%.

## SYMBOLS:

Used symbols and signs listed in the ISO standard 15223-1, refer to the Definition of Symbols document.