ZYMUTEST Anti–Protein Z IgM–Isotype

REF RK025B

Auto-antibodies to anti-Protein Z, IgM isotypes

FOR RESEARCH USE ONLY.

DO NOT USE IN DIAGNOSTIC PROCEDURES

HYPHEN BioMed

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INTENDED USE:

The ZYMUTEST anti-Protein Z, IgM, ELISA kit, is an enzyme immuno-assay designed for measuring auto-antibodies to Protein Z of the IgM isotype, in human plasma or in any biological fluid where auto-antibodies to Protein Z must be measured.

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

SUMMARY AND EXPLANATION:

The ZYMUTEST anti-Protein Z, IgM, specifically measures human autoantibodies to Protein Z of the IgM isotype, which are reactive with immobilised Protein Z. IgG or IgA isotypes are not measured.

PRINCIPLE:

The assay of human autoantibodies to Protein Z with the ZYMUTEST anti-Protein Z, IgM kit, is designed with highly purified human Protein Z coated onto a micro ELISA plate.

The diluted plasma sample or biological fluid is introduced into one of the microwells of the coated plate. When present, anti-Protein Z autoantibodies bind to immobilised Protein Z. Following a washing step, bound autoantibodies of the IgM isotype are revealed with a goat anti-human IgM (µ specific)-peroxidase conjugate, which reacts specifically with IgM isotypes. Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of hydrogen peroxide (H2O2), is introduced and a colour develops. The colour turns yellow when the reaction is stopped with sulfuric acid. The colour developed is directly proportional to the amount of anti-Protein Z autoantibodies, of the IgM isotype, present in the tested sample.

Tested samples:

- Trisodium citrate or Na2 EDTA anticoagulated human plasma or human serum.
- Any biological fluid where human autoantibodies to Protein Z, of the IgM isotype, must be assayed.

REAGENTS:

1. **COAT: Micro ELISA plate,** containing 12 strips of 8 wells, coated with highly purified human Protein Z, then stabilized; the plate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
2. **SD**: Two vials containing 50 ml of **Autoimmunity-Sample Diluent**, ready to use. Contains Sodium Azide.
3. **CAL**: Three vials of **anti- Protein Z, IgM, calibrator**, lyophilised. When restored with **1 ml** of **Autoimmunity Sample Diluent**, the ready to use calibrator is obtained (already diluted 1:100).
   This calibrator has a defined anti- Protein Z concentration, expressed in **Arbitrary Units (AU)** and indicated on the flyer provided with the kit.
4. **C–:** Three vials of **negative control**, lyophilised. When restored with **1 ml** of **Autoimmunity-Sample Diluent**, the ready to use negative control is obtained (already diluted **1:100**).
5. **IC:** Three vials of **immunoconjugate (Anti-IgM (µ specific)-HRP immunoconjugate)**, affinity purified goat antibodies specific for human IgM coupled to HRP, lyophilised.
6. **CD:** One vial of 25 ml of **conjugate diluent**, ready to use.
7. **WS:** One vial of 50 ml of 20 fold concentrated **Wash Solution**.
8. **TMB:** One vial of 25 ml peroxidase substrate: 3,3',5,5' **– Tetramethylbenzidine** containing hydrogen peroxide, ready to use.
9. **SA:** One vial of 6 ml of **0.45 M Sulfuric Acid (Stop Solution)**, ready to use.

Note: Use only components from a same kit lot number. Do not mix components from different lots when running the assay.

WARNINGS AND PRECAUTIONS:

- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* use is intended for professional use in the laboratory.

REAGENT PREPARATION:

1. **Micro ELISA plate:** open the plastic 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 **4 weeks** in their original aluminium pouch, in presence of the desiccant, hermetically closed and protected from any moisture, and stored in the provided microplate storage bag (minigrip).
2. **Autoimmunity-Sample Diluent:** It is ready to use. When open, it can be used for **4 weeks**, stored **at 2-8 °C**, and provided that any bacterial contamination is avoided during use. This reagent contains 0.05% Kathon CG.
3. **Calibrator:** restore each vial with **1 ml** Autoimmunity Sample Diluent in order to obtain the ready to use calibrator. It corresponds to a plasma containing IgM isotype auto-antibodies to Protein Z, already **diluted 1:100**. Following reconstitution, the calibrator is stable for **5 days at 2-8°C**, provided that any bacterial contamination is avoided during use.
4. **Negative control:** restore each vial with **1 ml** autoimmunity-sample diluent in order to obtain the ready to use negative control. It corresponds to a normal plasma, already **diluted 1:100**. Following reconstitution, the IgM negative control is stable for **2 weeks at 2-8°C**, provided that any bacterial contamination is avoided during use.

Warning: Protein Z used for coating the plates is extracted from human plasma. Negative control is also prepared with human plasma. Any human plasma used is tested with registered methods and found negative for HIV antibodies, HBs Ag and HCV antibodies. However, no assay may warrant the total absence of infectious agents. Any product of human origin must then be handled with all the required cautions, as being potentially infectious.

5. **Anti-IgM (µ specific)-HRP immunoconjugate**: each vial must be restored with **7.5 ml of conjugate diluent**. Let the pellet completely dissolve before use, and shake the vial gently in order to homogenize the contents. The restored conjugate is stable for at least **24 hours at room temperature** or for at least **4 weeks at 2-8°C**.
6. **Conjugate diluent**: It is ready to use. When open, it can be used for **4 weeks**, stored **at 2-8 °C**, and provided that any bacterial contamination is avoided during use. This reagent contains 0.05% Kathon CG.
7. **Wash Solution**: Incubate the vial for 15-30 minutes in a water bath, at 37°C, until complete dissolution of solids, when present. 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). The Wash Solution must be stored at 2-8°C in its original vial and used within **4 weeks following opening**. The diluted Wash Solution must be used within **7 days**, when protected from any contamination. This reagent contains 0.05% Kathon CG.
8. **TMB substrate:** It is ready to use. When open, it can be used for **4 weeks**, stored at **2-8°C**, and provided that any bacterial contamination is avoided during use.
9. **Stop solution:** Solution containing 0.45M Sulfuric acid: It is ready to use.

***Note*:** Bring the kit at room temperature, at least 30 min. before use. Store the unused reagents at 2-8°C.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Materials:

- **8-channel** or **repeating pipette** allowing dispensing 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.
- **Distilled water**.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI GP44-A4[6] (and CLSI H21-A5[7]) guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references.

PROCEDURE:

Assay method:

Plasma or serum is tested at the **1:100** dilution in autoimmunity-Sample Diluent. When high amounts of auto-antibodies to Protein Z are expected, samples must be assayed at **1:200** or **1:400** dilution. Results must then be multiplied by **2** or **4**.

The calibrator and the negative control are ready to use and correspond to plasmas **already diluted to 1/100**.

Calibration curve: The assay can be calibrated with the calibrator (**CAL**) included in the kit, and which concentration **(C)** is indicated in arbitrary units

(AU), on the flyer provided. Prepare the standard solutions for calibration by doing a serial two-step dilutions of the calibrator (**CAL**) in Autoimmunity Sample Diluent, from **1:1** to **1:32**. A concentration range from **C:1** to **C:32** is obtained.

Remove the required number of strips from the aluminium pouch, for the series of measures to be performed. Then 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
anti-Protein Z IgM Calibrator	**200 µl**	Introduce the : – **calibrator** or – **negative control** or – **diluted sample** or – **sample diluent** into the micro ELISA plate wells.
or Negative control	**200 µl**	
or 1:100 diluted sample	**200 µl**	
Or Sample diluent (blank)	**200µl**	
Incubate for 30 minutes at room temperature (18-25 °C) (a,b)		
Wash Solution (20 fold diluted in distilled water)	**300 µl**	Proceed to 5 successive washings using the washing instrument (**b**)
Conjugate (anti-IgM (µ specific) HRP immunoconjugate), restored with 7.5 ml of conjugate diluent)	**200 µl**	Immediately after washing, introduce the anti-IgM (µ specific)-HRP immunoconjugate into the microELISA plate wells.
Incubate for 30 minutes at room temperature (18-25 °C) (a)		
Wash Solution (20 fold diluted in distilled water)	**300 µl**	Proceed to 5 successive washings using the washing instrument (**b**)
TMB / H_2O_2 Substrate	**200 µl**	Immediately after the washing, introduce the substrate into the wells. **Note**: The substrate distribution, row by row, must be accurate and at exact time intervals **(c)**.
Let the colour to develop for **5 min**. at room temperature (18-25 °C) **(a).**		
0.45 M Sulfuric Acid	**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)**
Wait for **10 min**. in order to allow the colour to stabilize and measure absorbance at **450 nm (A450) (d)**. Substract the blank value.		

(a) Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro ELISA plate shaker can be used.
(b) 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 and reduce the reactivity plate. 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 damage coating and lower plate reactivity.
(c) For addition of the substrate, the time interval between each row must be accurate and exactly determined.
(d) For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used.

QUALITY CONTROL:

- Calibrator and controls provided in the kit allow validating the right performance of the assay.
- Expected A450 values for undiluted calibrator and the negative control can present variations from lot to lot but they always are:

P = A_{450} for 1:1 calibrator: ≥ 1.5 **N = A_{450} for negative control: ≤ 0.25**

In addition, concentrations obtained for negative control must be within the acceptance range indicated on the flyer provided in the kit. If negative control is out of this range check carefully the assay conditions and re-run the assay, if required.

RESULTS:

- Results are expressed according to the **A_{450}** values obtained for samples, controls and using the calibration curve.
- The calibration curve is obtained by plotting the anti-Protein Z concentration expressed in **AU** on the abscissae and the corresponding **A_{450}** on the ordinates (see model on the flyer). The anti-Protein Z, autoantibody concentration, of the IgM isotype, for the sample, tested at the standard **1:100** dilution, and expressed in AU, is directly deduced from the curve.
- When higher dilutions are used, (**i.e., D**), the concentration measured must be multiplied by the complementary dilution factor (i.e., **D:100** ; for example **x2** for **1:200** or **x4** for **1:400**).
- Alternatively, an ELISA software (i.e., Dynex, Biolise, etc.), can be used for the calculation of concentrations.

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

INTERPRETATION OF RESULTS:

A single and standardised calibrator is used for the assay calibration and the calibration range is prepared using a serial two-step dilution. This ensures a higher reliability of the assay, and a higher accuracy and reproducibility from lot to lot, and run to run, for the cut-off.

Negative range: The calibrator expressed in Arbitrary Unit (**AU**), is defined respectively to the upper limit of the normal range, which corresponds to the mean value obtained in a normal population plus 2 standard deviations (SD). By definition, this corresponds to **10 AU**. Therefore:

Negative range: < 10 AU/ml

Grey zone : **≥ 10 AU/ml to < 20 AU/ml**

Positive range: The positive range corresponds to the following anti-Protein Z autoantibody concentrations:

Positive range: ≥ 20 AU/ml

The positive range can be classified as follows:

Low positive: **≥ 20 to < 50 AU/ml**
Moderate positive: **≥ 50 to < 100 AU/ml**
High positive: **≥ 100 AU/ml**

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- If the washing step is not correctly performed, the negative control can produce a high absorbance value. In order to avoid non-specific colour development, check that the washing step is performed efficiently.

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