



ZYMUTEST DDimer

RK023A

(Complete ELISA kit for the assay of human DDimer)

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

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INTENDED USE:

The ZYMUTEST DDimer kit is a two-site enzyme immuno-assay for specifically measuring the Fibrin Degradation Products (DDimer) in plasma, or in any fluid where DDimer can be present. There is no interference of plasma Fibrinogen, thanks to the use of an ultra specific monoclonal antibody for DDimer capture.

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

ASSAY PRINCIPLE:

In a first step, the diluted tested plasma or biological fluid is introduced into a microwell coated with a highly purified monoclonal antibody specific for DDimer. When present, this analyte is captured onto the solid phase. Following a washing step, the immunoconjugate, which is a monoclonal antibody coupled to horse radish peroxidase (HRP), is introduced, and binds to another free epitope of immobilized DDimer. Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of hydrogen peroxide (H₂O₂), is introduced and a blue color develops. The blue color turns yellow when the reaction is stopped with sulfuric acid. The amount of color developed is directly proportional to the concentration of DDimer in the tested sample.

TESTED SAMPLE:

- Trisodium Citrate or Na₂ EDTA anticoagulated human plasma.
- Any biological fluid where DDimer must be measured.

REAGENTS:

1. **COAT: Micro ELISA plate**, containing 12 strips of 8 wells, coated with a highly purified murine monoclonal antibody, specific for DDimer, then stabilized; the plate is packed into an aluminium pouch hermetically sealed, in presence of a desiccant.
 2. **SD: 2 vials** containing 50 ml of **Sample diluent**, ready to use.
 3. **CAL: 3 vials of DDimer Calibrator** lyophilized. When restored with 2 ml of Sample diluent, allows obtaining the calibrator plasma at about 200 ng/ml of DDimer. The exact DDimer concentration is indicated on the flyer provided in the kit.
 4. **CI: 1 vial** containing 0.5 ml of lyophilized **plasma DDimer Control I High** (human plasma).
 5. **CIII: 1 vial** containing 0.5 ml of lyophilized **plasma DDimer Control II Low** (human plasma).
- Note:** The DDimer concentrations and acceptance ranges for controls and calibrator can vary from lot to lot, but are precisely indicated for each lot on the flyer provided in the kit.
6. **IC: 3 vials of Anti-(H)-DDimer-HRP immunoconjugate**, a monoclonal antibody coupled to HRP, lyophilized.
 7. **CD: 1 vial** of 25 ml of **Conjugate Diluent**, ready to use.
 8. **WS: 1 vial** of 50 ml of 20 fold concentrated **Wash Solution**.
 9. **TMB: 1 vial** of 25 ml peroxidase substrate: 3,3',5,5' - **Tetramethylbenzidine** containing hydrogen peroxide.
 10. **SA: 1 vial** of 6 ml of **0.45M Sulfuric acid (Stop solution)**.

Note:

Use only components from kits with a same lot number. Do not mix components from kits from different lots, when running the assay.

REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED:

- **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.

REAGENTS PREPARATION, STORAGE AND STABILITY:

In their original packaging box, before use, when stored at 2-8°C, the unopened reagents are stable until the expiration date printed on the box.

1. **Micro ELISA plate:** open the plastic pouch and take off the required amount 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. **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. **DDimer Calibrator:** restore each vial with **2 ml** of Sample Diluent. This solution is stable for at least **8 hours** at room temperature.
4. **Plasma DDimer Control I High** (human plasma, high): restore with **0.5 ml** distilled water, mix with vortex during 5 seconds or until complete dissolution of the content, let for 15 min at room temperature. Homogenize before use.
1. **Plasma DDimer Control II Low** (human plasma, low): restore with **0.5 ml** distilled water, mix with vortex during 5 seconds or until complete dissolution of the content, let for 15 min at room temperature. Homogenize before use.

Note:

when restored, DDimer controls are stable for **8 hours** at **room temperature**, **24 hours** at **2-8°C** or **2 months** frozen at **-20°C** or below.

Warning:

Plasma DDimer Calibrator (3) and controls (4&5) are prepared with normal human plasma. This latter was tested with registered methods and found negative for HIV antibodies, HBs Ag and HVC 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.

6. **Anti-DDimer-HRP immunoconjugate:** each vial must be restored with **7.5 ml** of **Conjugate Diluent**. Leave the pellet to be completely dissolved before use, and shake the vial gently in order to homogenize the content. The restored conjugate is stable for at least **8 hours** at **room temperature** or for at least **4 weeks** at **2-8°C**.
7. **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.
8. **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.
9. **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.
10. **Stop solution:** 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.

The stability studies at 30°C show that the reagents can be shipped at room temperature without damage.

PROCEDURE:

Specimen collection:

Blood (9 vol.) must be collected on 0.109M citrate anticoagulant (1 vol.) by a clean venipuncture; plasma supernatant is decanted following a **20 min.** centrifugation at **2,500 g**; citrated plasma should be tested within **4 hours** or stored frozen at **-20°C** or below for up to **6 months**, and thawed for 15 min. at 37°C just before use. Thawed specimen must be tested within **4 hours**.

EDTA collected human plasma may also be used.

Blood activation must be avoided during collection and plasma preparation in order to prevent from any DDimer generation ex-vivo.

Tested plasma or sample or controls:

The sample must be tested **fifty fold (1:50)** diluted in the Sample diluent. For expected DDimer concentrations **>10 µg/ml**, plasma or samples can be tested at a higher dilution, **1:100**, or **1:200**, or more. If low DDimer concentrations are expected, and if a high sensitivity is required, samples can be tested at a lower dilutions, i.e. **1:20**, **1:10** or **1:5**. Controls I and II must be tested **fifty fold (1:50)** diluted, with Sample Diluent.

Calibration:

Using the **DDimer calibrator (CAL)** provided in the kit, prepare the following standard solutions.

DDimer concentration	C	C/2	C/4	C/10	C/20	0
Vol. of Plasma DDimer calibrator	1 ml	0.5 ml	0.25 ml	0.1 ml	0.05 ml	0 ml
Vol. of Sample Diluent	0 ml	0.5 ml	0.75 ml	0.9 ml	0.95 ml	1 ml

Mix gently for a complete homogenization.

The standard dilutions are stable for at least **6 hours** at room temperature.

Assay procedure:

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 tested specimen or the reagents and perform the various assay steps as indicated on the following table:

Reagent	Volume	Procedure
DDimer Calibrator or tested sample or controls diluted 1:50 or -Sample Diluent (blank)	200 µl	Introduce the standard solutions, or the tested samples or the sample diluent, in the corresponding micro ELISA plate well.
Incubate for 1 hour 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).
Conjugate (anti DDimer monoclonal antibody coupled with peroxidase. Restored with 7.5 ml of conjugate diluent)	200 µl	Introduce the Anti-(H)-DDimer - HRP immunoconjugate in the micro ELISA plate wells (b).
Incubate for 1 hour 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 ₂ O ₂ Substrate	200 µl	Immediately after the washing, introduce the substrate into the wells. The substrate distribution, row by row, must be accurate and at exact time intervals (b, c).
Incubate for exactly 5 minutes at room temperature (18-25 °C) (a)		
0.45M Sulfuric Acid	50 µl	Following exactly the same time intervals than for the addition of substrate, stop the color development by introducing the 0.45M sulfuric acid (c).
Wait for 10 minutes in order to allow the color to stabilize and measure absorbance at 450 nm (A450) (d) . Subtract the blank values		

Note:

- a) Avoid letting the plate in the bright sunlight during incubations and more particularly during color 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. 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.
- c) 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.
- d) For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used.

Warning:

The calibrators and the tested samples must be distributed rapidly, in order to avoid differences in the assay kinetics between the different wells of the micro ELISA plate. It is recommended to distribute all the plate within less than 10 minutes.

RESULTS:

On a linear graph paper plot the DDimer concentrations on abscissae and the corresponding absorbances on ordinates (See the flyer included in the kit).

From the curve obtained, deduce the DDimer concentration for the tested sample. For obtaining the DDimer concentration in this sample, this value must be **multiplied by the dilution factor**, usually 50 (or by the dilution factor actually used 1:5, 1:10, 1:20, 1:100, 1:200, etc...).

For controls I and II, the concentrations measured must be multiplied by **50**.

Alternatively, an ELISA software (i.e. Dynex, Biolise, etc...) can be used for the calculation of concentrations.

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

BIOCHEMISTRY:

In presence of blood activation, thrombin and plasmin generation are stimulated together, which generates fibrin related products and fibrin degradation products, at various stages. In vivo, fibrin clot lysis generates very heterogeneous degradation products, usually with high molecular weight, including X-oligomers, D-X-D, Y-D and DDimer-E complexes. All these products are called DDimer. Actually, DDimer itself is rarely present in blood. The specific DDimer epitopes are exposed during fibrin degradation, and measured as DDimer. The DDimer concentration in normal human plasma is usually < 400 ng/ml.

ASSAY CHARACTERISTICS:

The Zymutest DDimer assay measures homogeneously the various Fibrin Degradation Products generated in vivo, when a fibrin clot is formed and degraded by the reactive Fibrinolysis, whether its degradation stage is. The assay does not react with fibrinogen.

LIMITATIONS OF THE ASSAY:

Any blood activation must be avoided ex-vivo, in order to keep the assay strictly specific for fibrin generation and degradation in vivo, and to avoid any overestimation of the DDimer measured concentration.

| *Changes compared to the previous version.*