ZYMUTEST uPA Antigen

 # RK013A

(One step ELISA method for the assay of Human Urokinase-type Plasminogen Activator Antigen)

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE:

The ZYMUTEST uPA kit is a one step, two-site immuno-assay for measuring human urokinase-Plasminogen Activator (uPA) in plasma, or in any fluid where uPA can be present.**This kit is for research use only and should not be used for patient diagnosis or treatment.**

ASSAY PRINCIPLE:

First, the immunoconjugate, which is a mixture of 2 monoclonal antibodies specific for uPA coupled to horse radish peroxidase (HRP), is introduced into the microwells coated with another monoclonal antibody specific for uPA (1). Then, the diluted tested sample is immediately introduced, and the immunological reaction starts. When present, uPA binds onto the monoclonal antibody coated solid phase through one epitope, and fixes the monoclonal antibodies coupled to HRP by other epitopes. Following a washing step, the peroxidase substrate, 3,3',5,5' – Tetramethylbenzidine (TMB), in presence of Hydrogen Peroxide (H_2O_2), is introduced and a blue colour develops. When the reaction is stopped with Sulfuric Acid, a yellow colour is obtained. The amount of colour developed is directly proportional to the concentration of human uPA in the tested sample.

TEST SAMPLE:

- Trisodium Citrate or Na_2 EDTA anticoagulated human plasma.
- Any biological fluid where uPA: Ag must be measured.

REAGENTS:

1. **COAT: Micro ELISA plate,** containing 12 strips of 8 wells, coated with a murine monoclonal antibody specific for human uPA, then stabilised; the plate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
2. **SD:** 2 vials containing 50ml of **F-Sample Diluent**, ready to use.
3. **Std:** 3 vials of **(h) uPA Standard**, lyophilised. Each vial, when restored with 1 ml of distilled water and diluted two fold with F-Sample Diluent, a solution containing of recombinant human uPA is obtained. The exact uPA-Ag concentration is indicated on the flyer provided in the kit.
4. **CI:** 1 vial containing **1 ml** of lyophilised **Plasma Control I (high) (UTA)** (human plasma).
5. **CII:** 1 vial containing **1 ml** of lyophilised **Plasma Control II (low) (UTA)** (human plasma).

Note*:* The uPA concentrations and acceptance ranges for controls can vary from lot to lot, and are indicated on the flyer provided in the kit.

6. **IC:** 3 vials of **anti-(h)-uPA-HRP immunoconjugate**, a mixture of 2 monoclonal antibodies coupled to HRP, lyophilised.
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. Ready to use.
10. **SA:** 1 vial of 6 ml of **0.45M Sulfuric acid** (Stop solution). Ready to use.

Note**:** Use only components from kits with the same lot number. Do not mix components from different lots of kits 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. **F-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. **(h) uPA Standard:** restore each vial with **1 ml of** distilled water. This solution is stable for at least **8 hours** at room temperature.
4. **Plasma Control I** (human plasma, **high, UTA**): restore with **1 ml** distilled water.
5. **Plasma Control II** (human plasma, **low, UTA**): restore with **1 ml** distilled water.

Note: when restored, uPA 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: Standards and Plasma uPA 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-(h)-uPA-HRP immunoconjugate**: each vial must be restored with **2 ml of Conjugate Diluent**. Let the pellet to be completely dissolved 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**.
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 preparing 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 and stored at **2-8°C**. 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.

    Cautions*:* Sulfuric acid, although diluted to 0.45M is caustic. As for any similar chemical, handle Sulfuric acid with great care. Avoid any skin and eye contact. Wear protection glasses and gloves when handling.

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

PROCEDURE:

Specimen collection:

Blood (9 vol.) must be collected on 0.109M citrate anticoagulant (1 vol.) ; plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; citrated plasma should be tested within 8 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.
In order to avoid diurnal variations, uPA should be tested preferentially on fasting samples, collected at morning.
EDTA collected human plasma may also be used. Conditions of storage are the same than those for citrated plasma.

Tested plasma or sample or controls:

The sample must be tested diluted **two fold (1:2)** in F-Sample Diluent. For expected uPA concentrations > 20 ng/ml, plasma or samples can be tested at a higher dilution**, 1:4, or more**. Undiluted samples can be used for expected low uPA: Ag concentrations.

Plasma Controls I and II must be tested diluted **two fold (1:2)**, with F-Sample Diluent.

Calibration:

Using the **uPA** standard provided in the kit, with the uPA: Ag concentration "**C**" indicated, for each lot of reagents, on the flyer provided in the kit, prepare the following standard solutions:

uPA concentration (ng/ml)	C	C/2	C/4	C/10	C/20	0
Vol. of uPA Standard	1 ml	0.5 ml	0.25 ml	0.1 ml	0.05 ml	0 ml
Vol. of F-Sample Diluent	0 ml	0.5 ml	0.75 ml	0.9 ml	0.95 ml	1 ml

Mix gently for a complete homogenisation.
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 reagents and perform the various assay steps as indicated on the following table:

Reagent	Volume	Procedure
Conjugate anti (h)-uPA-HRP (Restored with 2 ml of Conjugate Diluent)	**50 µl**	Introduce the Anti-(h)-uPA- HRP immunoconjugate in the micro ELISA plate wells
uPA Standard or tested sample or F- Sample Diluent (blank)	**200 µl**	Introduce **immediately** the standard solutions or the tested samples in the corresponding micro ELISA plate well
Mix gently on a plate shaker or manually and incubate for 1 hour at room temperature		
Wash Solution (20 fold diluted in distilled water)	**300 µl**	Proceed to 5 successive washings using the washing instrument. **(a)**
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 **(b, c)**.
Incubate for exactly 5 minutes at room temperature (18-25 °C) (c)		
0.45 M Sulfuric Acid (5)	**50 µl**	Following exactly the same time intervals than for the addition of substrate, stop the colour development by introducing the 0.45 M sulfuric acid **(b).**
Wait for **10 minutes** in order to allow the colour to stabilize and measure absorbance at **450 nm (A450)**. Subtract the blank value **(d)**.		

Note:

a. 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 immobilised components. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument musty be adjusted in order to wash the plates gently, and to avoid a too drastic emptying, which could lower plate reactivity.
b. 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.
c. Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro-ELISA plate shaker can be used.
d. For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used.

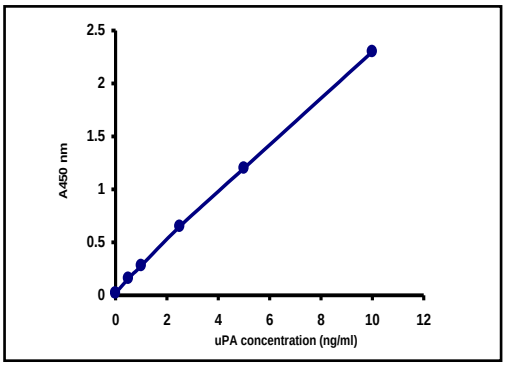
RESULTS:

On a linear graph paper plot the **uPA: Ag concentrations** on abscissae and the corresponding absorbances (A450) on ordinates.

From the calibration curve obtained, deduce the uPA: Ag concentration for the tested sample. For obtaining the uPA: Ag concentration in this sample, the value read on the calibration curve must be multiplied by the dilution factor **(i.e., 2, 4,...),** or directly if undiluted samples are used.
For controls I and II, the concentrations measured must be multiplied by **2**.
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.

EXAMPLE OF CALIBRATION CURVE:

The calibration curve below is an example only. Users must construct their own calibration curve, obtained using their standard dilutions.



BIOCHEMISTRY:

Urinary type Plasminogen Activator (uPA or urokinase) is a 55 Kd glycoprotein, synthesised mainly by kidney cells, by fibroblasts, by pneumocytes but also by tumour cells, as a single chain glycoprotein. It is activated into active High Molecular Weight-uPA, by various enzymes such as plasmin, kallicrein or cathepsins. uPA binds to its receptor uPA-R, present on some normal cells (phagocytic cells, fibroblasts) and on many tumour cells. uPA may also bind to soluble forms of uPA-R. uPA promotes fibrinolysis by converting plasminogen to plasmin. The normal uPA: Ag concentration in normals is from 0 to 5 ng/ml. The major inhibitor of uPA is PAI-1. In blood, the half-life of uPA is of about 8 min. in humans.

ASSAY CHARACTERISTICS:

This monoclonal antibody based assay, has homogeneous reactivity to the various forms of uPA (HMW-uPA, LMW-uPA, and uPA complexed with PAI-1).

REFERENCES:

1. van Boheemen P.A., van den Hoogen C.M., Koolwisk P.: Comparison of the inhibition of Urokinase-type Plasminogen Activator (uPA) Activity by Monoclonal antibodies specific for uPA as Assessed by different Assays: Fibrinolysis, 1995, 9, 343-349.