TECHNOZYM® u-PA ELISA Kit

For research use only REF TC12010

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Human urokinase (u-PA) is an enzyme synthesized by and released from numerous cell types which functions as an activator of the fibrinolytic enzyme system. Its ability to lyse fibrin clots makes it useful as an effective thrombolytic agent in the management of a variety of disease states including pulmonary embolism and localized thrombosis. Several malignant tumors, especially those of the urogenital and gastrointestinal tracts, have been shown to produce increased quantities of urokinase.

APPLICATION

The u-PA ELISA can be used to detect elevated u-PA antigen levels. The u-PA antigen ELISA can also be used for determination of u-PA antigen in tissue extracts.

TEST PRINCIPLE

The TECHNOZYM u-PA test is a solid phase enzyme immunoassay.

SPECIFICITY

The TECHNOZYM u-PA ELISA measures single and double-chain urokinase as well as urokinase-serpin complexes. It is unaffected by and does not recognize other plasminogen activators. This test system measures u-PA quantities from 0.6-10 ng/mL. The normal u-PA value in plasma is 1.8 ± 0.6 ng/mL. Inter- and intra-assay variations are less than 10 % and 5 %, respectively.

TEST SAMPLES

Use fresh EDTA or citrated plasma samples. The total time between blood collection and plasma freezing should not exceed 90 min. Thawing and refreezing of plasma aliquots is not recommended. Thawing for assay is achieved rapidly using a water bath at 37° C. After thawing, place plasma samplesin a crushed ice-water mixture until analysis. U-PA antigen can also be determined in serum; the values obtained do not differ significantly from those determined in plasma.
Haemolytic and lipemic plasma may be used. Plasma samples cannot be used if any evidence of coagulation is seen. If only citrated plasma is available for testing, EDTA must be added to a 5 mM end-concentration before testing (1.86 mg EDTA/mL plasma).

KIT COMPONENTS

1. **PLATE + PLATE COVER**
   12x8 well microtiter strips precoated with a monoclonal anti-u-PA antibody and blocked with 1 % bovine serum albumin (BSA), lyophilised.
2. **STANDARD**
   1x lyophilized u-PA, calibrated against the WHO International Standard for High Molecular Weight Urokinase
   Concentrations are lot-dependent; consult batch table or label on the vial
3. **BIOTINYLATED DETECTING ANTIBODY** (purple cap)
   1x Biotinylated polyclonal anti human urokinase antibody (concentrated)
4. **STREPTAVIDIN-HRP SOLUTION** (brown cap)
   1x Streptavidin conjugated to HRP (horseradish peroxidase) (concentrated).
5. **POX DILUTION BUFFER** – (white cap)
   2x 12ml PBS, 1% BSA Ready to use
6. **DILUTION BUFFER –** (white cap)
   1 x 20 ml 2.5x concentrated (PBS, 1 % BSA, 20 mM EDTA, 10 KIU/ml aprotinin, 20 mM Benzamidine)
7. **SUBSTRATE -** (green cap)
   1x 12 mL TMB (Tetramethylbenzidine) in substrate buffer containing H_2O_2. Ready to use.
8. **STOP SOLUTION -** (red cap)
   1x 15 mL 0.5 M Sulphuric Acid. Ready to use
9. **WASHING BUFFER – concentrate**
   1x80 ml (PBS pH 7,3) containing detergent, 0,01 % merthiolate

Kit storage: Store all components at 2... 8 °C.

ALSO REQUIRED

1. Micropipettes and a multichannel micropipette; pipette tips.
2. Glass or plastic test tubes for diluting the standard + samples.
3. Laboratory bottles or beakers and graduated cylinders for diluting wash and dilution buffer.
4. Distilled or deionised water.
5. Absorbent paper towels.
6. Microtiter plate washer (alternatively, washing can be performed manually using a multichannel pipette or repeating syringe).
7. A microtiter reader equipped with a 450 nm filter and, if possible, a 620 nm reference filter.
8. A 37 ° C incubator
9. Graph paper.

REAGENT HANDLING & BENCH STABILITY

All components contained in the kit may be used until the expiry date as indicated. The shelf life of the components after opening, reconstitution and/or dilution may be inferred from the table below:

Material/Reagent	State	Storage	Stability
calibrator	after reconstitution	-20 °C	6 months
ELISA test strips	after opening	2 ... 8 °C with adhesive film in plastic bag with drying agent	expiry date
Washing buffer concentrate	after opening	2 ...8 °C	6 months
Washing buffer	Dilution 1+11.5 of concentrate	2 ... 8 °C	3 weeks
POX Dilution buffer & Dilution buffer	after opening	2 ... 8 °C	6 months
Biotinylated antibody & Streptavidin-HRP	after opening	2 ... 8 °C	6 months
	working solution	room temperature	60 minutes
Substrate & Stop solutions	after opening	2 ... 8 °C	expiry date

Preparation of reagent

1. Before starting the test, all the required components are to be brought to room temperature.
2. Preparing the washing buffer: Dilute 1 part by volume washing buffer concentrate with 11.5 parts by volume distilled water (1+11.5). Mix well (Diluted washing buffer concentrate = washing buffer). There may be crystalline precipitations which will dissolve at 37 °C within 10 minutes.
3. Preparing the Dilution buffer: Dilute 1 part by volume dilution buffer concentrate with 1.5 parts by volume distilled water (1+1.5). Mix well
4. Reconstituting calibrator:
   The calibrator is reconstituted with 500 µl distilled water and mixed for 10 seconds after a reconstitution time of 15 minutes (vortex mixer). Reconstituted components are clear to slightly turbid.
5. Biotinylated Detecting Antibody: Preparing the working solution (1+40)

For 8 test wells: **25 µl Biotinylated Ab with 1000 µl POX dilution buffer.**

6. Streptavidin-HRP: Preparing the working solution (1+10)

For 8 test wells: **100 µl Streptavidin-HRP with 1000 µl POX dilution buffer.**

STANDARD CURVE
Preparation of calibrators for standard curve see batch table.

SAMPLE DILUTIONS
Run plasma undiluted.

Abnormally high plasma (i.e. samples collected during u-PA therapy) dilute
1:2 150 µL plasma + 150 µL dilution buffer
or 1:4 100 µL plasma + 300 µL dilution buffer

Time table Summary of procedure		time required	Temp.
Reagent, standard sample handling		1-2 hours	
1. Sample - incubation	100 µL	16-24 hours	4 °C
wash 3 times	250 µL		
2. Biotinylated Detecting Ab – incubation	100 µL	1 hour	37 °C
wash 3 times	250 µL		
3. Streptavidin-HRP – incubation	100 µL	20 minutes	37 °C
wash 3 times	250 µL		
4. Substrate – incubation	100 µL	15 minutes	RT
Stop solution	100 µL		
Read absorbances			

ASSAY PROCEDURE

1. **SAMPLE/ STANDARD ADDITION**
Pipette 100µL of the diluted samples/standard into separate wells. Blank well is filled with 100µL dilution buffer. Running standard/sample in duplicate is recommended.
2. **SAMPLE INCUBATION**
Cover the plate with a plastic foil and incubate 16-24 hours at 4 °C.
3. **WASH PLATE**
Wash three times: by adding 250 µL of wash buffer to each well and tip out the contents. Wash the strips twice further with wash buffer. Tap strips on absorbent paper and make sure the wells are completely dry
4. **BIOTINYLATED DETECTING ANTIBODY ADDITION**
Add 100 µL of the diluted biotinylated detecting antibody to each well, preferably with a multichannel pipette.
5. **BIOTINYLATED DETECTING ANTIBODY INCUBATION**
Cover and incubate the plate for 1 hour at 37 °C.
6. **WASH PLATE**
Wash three times as described in step 3.
7. **STREPTAVIDIN-HRP ADDITION**
Add 100µL of the diluted streptavidin-HRP to each well, preferably with a multichannel pipette
8. **STREPTAVIDIN-HRP INCUBATION**
Cover and incubate the plate for 20 minutes at 37° C
9. **WASH PLATE**
Wash three times as described in step 3

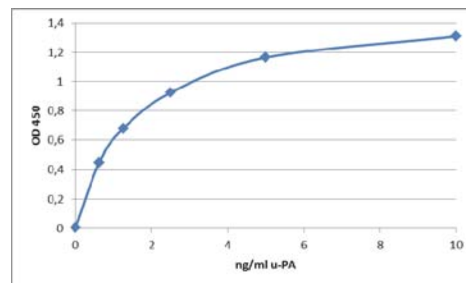
8\. **SUBSTRATE**
Pipette 100 µL of TMB substrate to each well. Incubate for 15 minutes at room temperature.

9\. **STOP**
Pipette 100µL of stop solution to all wells.

10\. **READ**
Measure absorbances at 450 nm (with 620 nm reference filter if available). Read absorbances within one hour after the addition of the stop solution.

10\. **GRAPH**
Construct a graph of standard curve.

11\. Locate the absorbance for each sample on the curve and read the corresponding value from the horizontal axis. Do not forget to multiply by the dilution factor (2) or (4) for the diluted samples.



EVALUATION OF RESULTS

Plasma u-PA values within the normal range (1.8 ± 0.6 ng/mL) will be detectable at the lower end of the standard curve where exact values in nanograms cannot be precisely determined. When testing plasma with increased u-PA levels, it may be necessary to dilute the plasma in dilution buffer to obtain measurable results. Remember to multiply the u-PA value by the dilution factor.

LITERATURE

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