

# TintElize® tPA

Reagents for up to 96 tests

For *in vitro* diagnostic use only

## FOR INFORMATION USE ONLY

**Not to be used for performing the assay.**

**Refer to the insert accompanying kit**

### I. INTENDED USE

TintElize® tPA is intended for quantitative determination of human tissue plasminogen activator antigen (tPA) in plasma by enzyme immunoassay. The clinical utility of the assay is to detect disorders of the fibrinolytic system.

### II. SUMMARY

Tissue-type plasminogen activator (tPA) constitutes an important agent in the fibrinolytic pathway and the levels of tPA are thought to have a major effect on fibrinolytic potential<sup>1</sup>. The physiological role of tPA is to activate plasminogen to plasmin, which in turn degrades fibrin to soluble degradation products. Two forms of the tPA molecule are present in plasma, the single-chain and the two-chain form, and both forms are equally capable of activating plasminogen. Fibrinolysis is regulated by specific molecular interactions between tPA and fibrin as well as between plasmin and the specific plasmin inhibitor,  $\alpha$ -2-antiplasmin<sup>1</sup>.

### III. PRINCIPLE

The Biopool TintElize® tPA utilises the double antibody principle<sup>2,3,4,5</sup>. Plasma sample or standard containing tPA is added to a microtest well which is coated with goat anti-tPA IgG and contains soluble non-immune goat IgG. After an incubation sufficient to allow >95% of the tPA to bind to the capture antibodies, HRP-labelled Fab fragments of anti-tPA IgG are added. These are allowed to react with the bound tPA. The wells are emptied and washed to remove unbound conjugate after which peroxidase substrate (OPD/H<sub>2</sub>O<sub>2</sub>) is added. The amount of yellow colour developed is directly proportional to the amount of tPA present in the sample. The tPA antigen standard, provided with the TintElize tPA, contains human single-chain tPA and is calibrated against the international standard for tPA, lot 86/670 distributed by NIBSC, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, England<sup>6,7</sup>.

### IV. REAGENTS

#### A. Reagent Descriptions

- Microtest strips**, 6 pcs:  
Framed 16-well strips, precoated with goat anti-tPA IgG and prefilled with non-immune goat IgG and indicator dye.
- Standard 0 ng/ml**, 3 vials:  
Lyophilised human plasma depleted of tPA. Contains merthiolate as preservative.
- Standard 30 ng/ml**, 3 vials:  
Lyophilised human plasma enriched with tPA. Contains merthiolate as preservative.
- Conjugate**, 1 vial:  
Lyophilised HRP-labelled anti-human tPA Fab fragments.
- PET-buffer**, 1 vial:  
PBS-EDTA-Tween 20 buffer substances sufficient to make 1 L solution.
- Substrate**, 1 vial:  
Lyophilised 1,2 phenylenediamine dihydrochloride (OPD) at 4.17% in buffer salts.
- Hydrogen peroxide**, 1 vial:  
2 ml, 0.15% H<sub>2</sub>O<sub>2</sub> in water.

### B. Reagent Preparation

- PET-buffer** Dissolve the content of the PET-buffer vial in 1 L water (use a magnetic stirrer for about 15 min.). This buffer is used to fill and wash the strips, and to reconstitute the conjugate.
- Standard 0 ng/ml and 30 ng/ml.** Add 0.5 ml of water to each vial, gently agitate for 5 minutes to completely dissolve contents. Mix the standard 30 ng/ml and standard 0 ng/ml in small test tubes in the following proportions below.  
**Warning:** See WARNINGS AND PRECAUTIONS.

Concentration (ng/ml)	Standard 30 ng/ml (µl)	Standard 0 ng/ml (µl)
0	0	200
10	75	150
20	150	75
30	200	0

- Microtest strips** Mark the top and bottom of each strip to prevent mix-ups.
- Conjugate** Add 7 ml PET-buffer directly to the conjugate vial and agitate gently for 5 minutes.
- Substrate** Dissolve in 2 ml of water and agitate 10-15 minutes to prepare substrate concentrate. Prepare OPD/H<sub>2</sub>O<sub>2</sub> substrate within 30 minutes before substrate addition.  
For one 16 well strip: Mix 300 µl substrate concentrate with 1.5 ml water and 300 µl hydrogen peroxide in a clean container.  
For the complete kit: Add 10 ml of water to the already dissolved 2 ml concentrate and shake for 4-6 minutes. Add all of the H<sub>2</sub>O<sub>2</sub> to the vial and mix. **Warning:** See WARNINGS AND PRECAUTIONS.
- Hydrogen peroxide ( H<sub>2</sub>O<sub>2</sub> )** Transfer to a clean capped 2-5 ml test tube.
- Stop solution (1.6 M H<sub>2</sub>SO<sub>4</sub>)** Add 2.5 ml concentrated sulphuric acid to 25 ml of water, totally 27.5 ml. **Warning:** See WARNINGS AND PRECAUTIONS.

### V. STORAGE AND STABILITY

The unopened reagents are stable until expiry date, indicated on the label, when stored at 2-8°C.

- PET-buffer** Store at 2-8°C and use within one month.
- Standard 0 ng/ml and 30 ng/ml.** Store on ice and use within 8 hours.
- Microtest strips** Store tightly sealed in the bag at 2-8°C for one month.
- Conjugate** Store dark at -20°C for one month. If frozen, thaw at room temperature before re-use. May be thawed and refrozen several times.
- Substrate** Store at -20°C for one month. May be thawed and refrozen.
- Hydrogen peroxide ( H<sub>2</sub>O<sub>2</sub> )** Store in the dark at 2-8°C for one month.
- Stop solution (1.6 M H<sub>2</sub>SO<sub>4</sub>)** Store in a glass bottle at ambient temperature (20-25°C).

### VI. WARNINGS AND PRECAUTIONS

The Standards are of human origin. Each donor unit of source plasma used in these products has been tested and found negative for Hepatitis B antigens, HIV I and II antibodies, Hepatitis C antibodies, syphilis antibodies and H.T.L.V. I/II antibodies by FDA approved methods. Because no test method can offer complete assurance that no infectious agents are present, these plasmas should be handled as patient plasma at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/ National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.

OPD and hydrogen peroxide are harmful and must be handled with care. Avoid ingestion, skin and eye contact. Wear glasses and gloves. Concentrated H<sub>2</sub>SO<sub>4</sub> is corrosive, use gloves and glasses. All wastes containing biological material should be properly labelled and stored separately from other wastes. Dispose of all waste materials according to prescribed international, national and local regulations.

The test should be used in conjunction with clinical observations and results of other laboratory tests.

## VII. SPECIMEN COLLECTION

Nine volumes of blood are collected in one volume of 0.1M trisodium citrate or 99 volumes of blood in 1 volume of 0.5M EDTA, followed by centrifugation at 2500 X g for 15 minutes. See NCCLS Document<sup>14</sup>. Human plasma samples can be collected with either citrate (0.1M) or with EDTA (0.5M). A plasma sample collected in citrate, containing 15 ng/ml tPA, was frozen at -20°C and thawed at 37°C five times with gentle agitation after each thaw. Assay after the fifth thaw gave about 14 ng/ml tPA. Plasma samples, collected in citrate, containing about 4 and 10 ng/ml tPA, retained these values after 2 years of storage at -20°C.

## VIII. PROCEDURE

### A. Reagents/materials provided:

- Microtest strips, 6 pcs:
- Standard 0 ng/ml, 3 vials:
- Standard 30 ng/ml, 3 vials:
- Conjugate, 1 vial:
- PET-buffer, 1 vial:
- Substrate, 1 vial:
- Hydrogen peroxide, 1 vial:
- Reagent Reservoirs, 6 pcs:

### B. Reagents and equipment required but not provided:

- Pipette 8-channel or repeating for 50-200 µl
- Pipettes 1-channel covering 20-1000 µl
- Water purified (glass distilled or water for injection)
- Sulphuric acid concentrated
- Microtest Plate Spectrophotometer operable at 492 nm
- Magnetic stirrer and stir-bar
- Microtest Plate Shaker, orbital movement 3-5 mm, speed 600 rpm.
- Squeeze bottle
- Paper towels or thin sponge
- Small plastic tubes (2-5 ml)

### C. Assay procedure

**Note!** Perform assay steps at ambient temperature (20-25°C). Temperature equilibrate all reagent solutions.

#### 1. Reconstitution of wells

Add 50 µl PET-buffer to each well using an 8-channel pipette (or a repeating pipette). Agitate gently for 1 minute.

#### 2. Standard and sample incubation

Add 20 µl of tPA standards (0, 10, 20, and 30 ng/ml) and 20 µl of each test sample (neat plasma) to the wells. **Note:** if venous occlusion plasma is assayed it may be necessary to dilute plasma 1:2 with tPA or tPA/PAI depleted plasma before use. Record the positions. Incubate the strips for 1 hour on a microtest plate shaker (see equipment list above for rpm value). A colour shift will take place in the wells shortly after addition of plasma samples indicating that an addition has been made. **Warning:** See Warnings and Precautions.

#### 3. Conjugate incubation

Add 50 µl of the conjugate to the wells using a repeating or an 8-channel pipette. Incubate the plate for 15 minutes on a microtest plate shaker.

#### 4. Wash

Discard the contents and wash the strips four times. Each wash is performed as follows: fill the wells completely with PET-buffer, use a squeeze bottle if convenient. Empty and then "dry" by hitting the strips 4-5 times, face down, against absorbing material (sponge or paper towels). Use gloves.

#### 5. Substrate incubation

Add 100 µl OPD/H<sub>2</sub>O<sub>2</sub> substrate to each well. Use 8-channel pipette. Incubate for 15 minutes on a microtest plate shaker. **Warning:** See Warnings and Precautions.

#### 6. Stop

Add 100 µl Stop solution to stop the enzymatic reaction. Add in the same order and with the same speed as the substrate was added. Agitate the plate for 5 minutes on a micro-test plate shaker to allow complete mixing and stabilisation of colour. If stored dark, the coloured product is stable for at least 2 hours. **Warning:** See Warnings and Precautions.

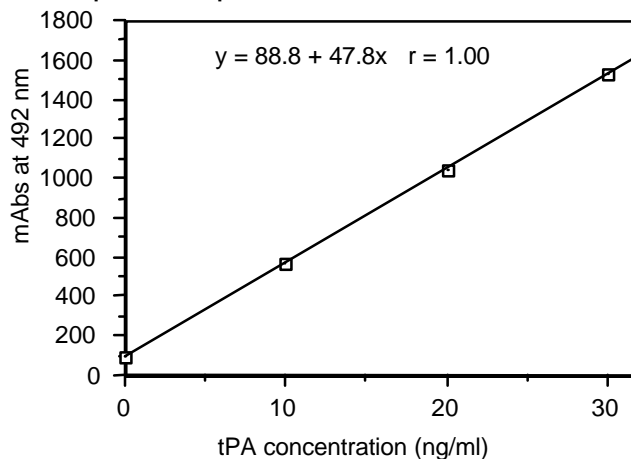
#### 7. Measurement

Set the absorbance at 492 nm in a microtest plate spectrophotometer. "Blank" the microtest plate reader against air; some reader manufacturers specify this as "no reagent blank". Measure the absorbance in all wells at A<sub>492</sub>.

## IX. CALIBRATION

Calculations: Plot A<sub>492</sub> against each 0, 10, 20, and 30 ng/ml standard. Fit a straight line to the points by a minimal least squares procedure. The tPA antigen in the patients plasma specimen can be determined by interpolation from the calibration curve. Results from samples diluted 1:2 with tPA or tPA/PAI depleted plasma should be multiplied by 2 for actual tPA concentration.

### Example of a sample calibration curve



## X. QUALITY CONTROL

It is recommended that 2 plasma samples containing between 4-10 ng/ml tPA antigen (low control) and 12-20 ng/ml tPA antigen (high control) be stored at -20°C or below, in small aliquots and used as quality control standards each time the assay is run. Failure to obtain a tPA antigen level within two standard deviations of the mean for each control standard may invalidate the assay. The Standards 0 ng/ml and 30 ng/ml are provided to construct the calibration curve and must not be used to check device performance.

## XI. LIMITATIONS OF PROCEDURE

1. Bacterial contamination results in peroxidase activity in the water (use the purest water available).
2. Use reagents equilibrated at ambient temperature, 20-25°C to minimise "edge effects", i.e. erroneous absorbance in the peripheral wells. Make certain the plate shaker does not heat the strips. If the top of the shaker feels warm to the hand, cover with a 1 cm thick sheet of insulation, e.g. Styrofoam.
3. It is extremely important to remove unbound conjugate before adding the substrate. Be sure that the wash volume completely fills the wells, and that the wells are completely emptied after each wash. Do not leave the empty wells to dry, fill with the next solution without delay.
4. Samples should be well mixed during thaw in a 37°C water bath.

## XII. INTERFERENCES

Heparin (<10 U/ml) does not influence the determination in plasma containing 12 ng/ml tPA. Urokinase (<8 ng/ml) does not influence the determination in plasma containing either 12 or 22 ng/ml tPA.

## XIII. EXPECTED VALUES

Healthy males and females, age 25-34 years, display median plasma tPA levels of 5.5 ng/ml and 4.0 ng/ml, respectively. Reference limits, 2.5 and 97.5 percentile, are about 1 ng/ml and 20 ng/ml. tPA antigen increases with age; at 55-64 years, the median levels are 8.6 ng/ml and 7.6 ng/ml for males and females<sup>8,9</sup>. In a study of 231 men who suffered myocardial infarction, the concentration of tPA antigen was significantly ( $p < .0008$ ) higher in the MI cases than the matched controls<sup>10</sup>. High tPA antigen levels have also been associated with future strokes<sup>13</sup>. Users should determine their own normal range of tPA antigen for local subjects.

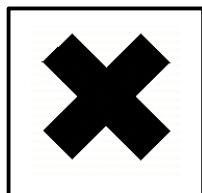
## XIV. PERFORMANCE CHARACTERISTICS

**A. Specificity and accuracy:** The accuracy of TintElize<sup>®</sup> tPA was shown in a study which compared tPA antigen and activity levels using the Biopool Spectrolyse<sup>®</sup>/fibrin tPA kit. Single-chain tPA was added to tPA depleted plasma in 31 samples and assayed by both methods. The linear correlation coefficient ( $r$ ) found was 0.99. The assay is unaffected by rheumatoid factor(s) and antibodies against goat IgG in the sample<sup>8,11</sup>. This is due to use of affinity purified antibodies as capture antibody, HRP conjugated Fab fragments of affinity purified antibodies as conjugate and large excess of non-immune goat IgG in each well. The immunoreactivities of single-chain and two-chain tPA in complex with  $\alpha_2$ -AP, PAI-1, and PAI-2 are >85% compared to non-complexed tPA<sup>12</sup>.

**B. Sensitivity and precision:** The calibration curve is linear between 0 and 30 ng/ml (0 to 0.6 ng tPA per well). For plasma samples the intra-assay (within run) and inter-assay (between run) precisions for a given device lot were:

tPA level (ng/ml)	COEFFICIENT OF VARIATION (C.V.)	
	% within run (n=48)	% between run (n=10)
6	5.5	3.5
15	4.9	5.4

## XV. RISK AND SAFETY



Xn

**Harmful – OPD**

R20/21/22: Harmful by inhalation, in contact with skin and if Swallowed.

R40: Limited evidence of a carcinogenic effect.

R52/53: Harmful to aquatic organisms, may cause lone-term adverse effects in the aquatic environment.

S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection.

S61: Avoid release to the environment. Refer to special instructions/safety data sheets.

## XVI. REFERENCES

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- Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays, NCCLS Document H21-A2, Vol. 11, No. 23, 1991.

## XVII. KEY GUIDE TO SYMBOLS



Use by



Lot



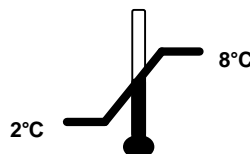
Catalogue number



Manufacturer



For *in vitro* diagnostic use



Store at 2-8°C



Consult accompanying documents



Biological risks

**Recon.**

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