

Chromolize™ tPA Assay Kit

Strip-well format. For the quantitative determination of human tissue plasminogen activator activity in plasma.

For *in vitro* diagnostic use.

FOR INFORMATION USE ONLY
Not to be used for performing the assay.
Refer to the insert accompanying kit

I. INTENDED USE

Biopool's Chromolize™ tPA is a bio-functional immunosorbent assay (BIA) intended for the quantitative determination of human tissue plasminogen activator activity in plasma. The clinical utility of the assay is to detect disorders of the fibrinolytic system.

II. SUMMARY

Tissue-type plasminogen activator (tPA) constitutes an important protein in the fibrinolytic pathway, and its activity is thought to play a major role in the fibrinolytic system^(1,4). The physiological role of tPA is to activate plasminogen to plasmin, which degrades fibrin to soluble fibrin degradation products (FDP). The tPA molecule is present in either the single-chain or two-chain form with similar plasminogen activating potentials. Fibrinolysis is regulated by specific interactions between tPA and fibrin, as well as between plasmin and the specific plasmin inhibitor, α_2 -antiplasmin.

In the assay of tPA, the fast-acting specific inhibitor for single-chain tPA, plasminogen activator inhibitor type 1 (PAI-1)^(5,6), is usually present in large excess over tPA and must be inhibited from quenching tPA activity. This is accomplished using Stabilyte™ blood collection tubes, which provide mild acidification and stabilisation of the sample⁽⁷⁾.

III. PRINCIPLE

The Biopool Chromolize™ tPA has been briefly described⁽⁸⁾. The sample tPA is captured by antibodies on the microtest wells. The SP-322 monoclonal antibody used allows excellent tPA recovery at pH 5.9 without inhibiting tPA activity. After the test plasma is discarded, the wells are washed with mild detergent. The tPA substrate consisting of plasminogen, a plasmin-sensitive chromogenic substrate, and tPA activity promoters, all in HEPES buffer pH 8.5, is added and the microtest wells are incubated at ambient temperature (18-25°C) for 90 minutes with agitation. Samples are read at 405 nm. The amount of colour developed is proportional to the amount of tPA activity in the sample. The tPA activity standard provided contains human single-chain tPA and is calibrated against the international standard for tPA, lot 86/670 distributed by NIBSC, South Mimms, Potters Bar, Hertfordshire, UK^(9,10).

IV. REAGENTS

A. Reagent Description

- 12 Framed Microtest Strips:**
Ready-to-use, lyophilised, 8-well strips coated with monoclonal antibody SP-322 directed against tPA.
- PET Buffer:**
Phosphate, NaCl, EDTA, and Tween 20 buffer concentrate sufficient for 1 L solution.
- tPA Activity Standard, 3 x 0.5 ml:**
Lyophilised human plasma containing 2.0 IU/ml human melanoma tPA.
- Substrate Reagent, 6 ml:**
Lyophilised H-D-But-CHT-Lys-pNA and poly-D-lysine.
- Plasminogen Reagent, 6 ml:**
Lyophilised plasminogen and FDP.
- Citrate Buffer, 8 ml:**
Ready-to-use citrate buffer, pH 5.9.
- HEPES Buffer, 13 ml:**

B. Reagent Preparation

- Microtest Strips:** Mark the top and bottom of each strip with indelible ink to prevent mix-ups.
- PET Buffer:** Dissolve contents of the PET Buffer vial in 1.0 L of reagent grade water. (Use a magnetic stirrer for about 15 minutes). PET Buffer is used to wash the strips.
- tPA Activity Standard:** Reconstitute with 500 μ l of reagent grade water and gently agitate for 5 minutes. Mix tPA Activity Standard and Citrate Buffer in small test tubes as below.

Concentration (IU/ml)	tPA Activity Standard (μ l)	Citrate Buffer (μ l)
0	0	150
0.5	50	150
1.0	100	100
1.5	150	50
2.0	150	0

- Substrate Reagent:** Reconstitute the vial with 6 ml HEPES Buffer and gently agitate for 5 minutes.
- Plasminogen Reagent:** Reconstitute the vial with 6 ml HEPES Buffer and gently agitate for 5 minutes.
- Stop Solution:** Add 1 ml of glacial acetic acid to 9 ml of water to obtain 1.7 M acetic acid. Discard excess and make fresh before each use.

V. STORAGE AND STABILITY

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

- Microtest Strips:** Store unused strips in a zip-lock bag at 2-8°C and use within 4 weeks.
- PET Buffer:** Store dissolved reagent at 2-8°C and use within 4 weeks.
- tPA Activity Standard:** Store reconstituted reagent on ice and use within 8 hours.
- Substrate Reagent** Store reconstituted reagent in the dark at -20°C for 4 weeks. May be thawed and refrozen twice.
- Plasminogen Reagent:** Store reconstituted reagent in the dark at -20°C for 4 weeks. May be thawed and refrozen twice.

VI. WARNINGS AND PRECAUTIONS

The Plasminogen Reagent and tPA Activity Standard 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. However no test can offer complete assurance that products derived from human blood will not transmit infectious disease. As with all materials of human origin, this product should be handled as a potentially infectious agent. 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

Plasma should be collected in the Biopool Stabilyte™ tubes with the same precautions and in a similar proper manner as with the other blood collection devices. Nine volumes of blood (4.5 ml) are collected in one volume of 0.5 M citrate, pH 4.3, followed by centrifugation at 2500 X g for 15 minutes. Remove plasma to a plastic test tube. See "Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays", NCCLS Document H21-A2, Vol. 11, No. 23, 1991. Plasma collected by this method may be stored at ambient temperature for up to 5 hours, or frozen at -20°C for up to 4 weeks or for up to 5 months at -70°C prior to assay.

VIII. PROCEDURE (MANUAL)

A. Material Provided:

- Microtest Strips
- PET Buffer
- tPA Activity Standard
- Substrate Reagent
- Plasminogen Reagent
- Citrate Buffer
- HEPES Buffer

B. Additional Material Required (but not provided):

- Reagent grade water
- Squeeze bottle
- Adjustable pipettes for 20-500 μ l
- Repeating pipette for 50 and 100 μ l
- Paper towels or thin sponge
- Small plastic tubes (2-5 ml)
- Magnetic stirrer and stir-bar
- Glacial acetic acid
- Microtest plate shaker with a uniform horizontally circular movement of 3-5 mm (e.g., IKA-Schuttler, Janke & Kunkel GmbH & Co. KG).
- Microtest plate spectrophotometer capable of measurements at 405 nm and 492 nm.

Perform assay steps at ambient temperature. Temperature equilibrate all reagent solutions prior to use.

1. Standard and Sample Incubation

Add 100 μ l of tPA standards (0, 0.5, 1.0, 1.5, 2.0 IU/ml) and 100 μ l of sample to the microtest strip wells. Record the positions. Incubate the strip(s) for 20 minutes on a microtest plate shaker at ambient temperature at 600 rpm. If venous occlusion plasma is assayed, it may be necessary to dilute the plasma 1:5 with Citrate Buffer to bring tPA activity into the assay range.

2. Wash

Discard the contents by tapping onto an absorbent towel or sponge and wash the strip(s) 4 times. Each wash is performed as follows: Fill the wells completely with PET Buffer using a squeeze bottle. Empty and "dry" by hitting the strip(s) 4-5 times, face down, against absorbing material (sponge or paper towels).

3. Substrate Incubation

Add 50 μ l Substrate Reagent to each well using the repeating pipette, then add 50 μ l Plasminogen Reagent to each well using the repeating pipette. Complete the Plasminogen Reagent addition within 2 minutes. Incubate for 90 minutes at ambient temperature on a microtest plate shaker at 600 rpm.

4. Stop

Add 50 μ l Stop Solution to each well and mix on a microtest plate shaker for at least 15 seconds. The yellow coloured reaction is stable for 1 hour at ambient temperature.

5. Measurement

Set the absorbance at 405 nm on the spectrophotometer. "Blank" the spectrophotometer against air. Some spectrophotometer manufacturers specify this as "no reagent blank". Measure the absorbance in all wells at 405 nm. A second measurement may be made at 492 nm and these subtracted from the readings at 405 nm. Paranitroanilide absorbs light at 405 nm, whereas the absorbance due to turbidity is approximately equal at 405 nm and 492 nm. Therefore, absorbance at 492 nm is measured and subtracted to correct for background due to turbidity.

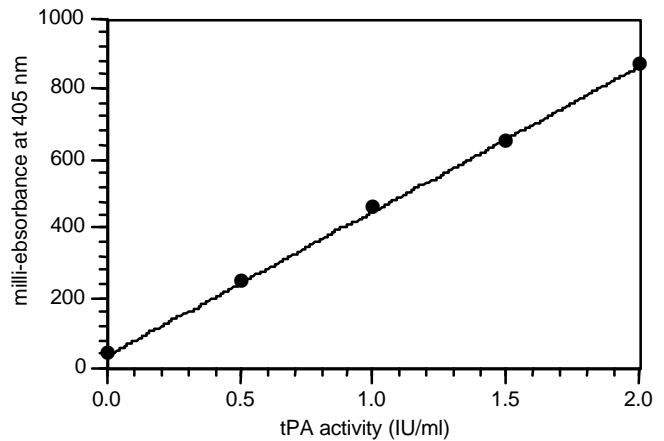
IX. CALIBRATION

The Chromolize™ tPA standard curve was correlated to the International tPA activity standard lot 86/670 (NIBSC), resulting in a correlation coefficient of 1.0 with a slope of 1.13.

Plot A_{405} or $A_{405}-A_{492}$ against each 0, 0.5, 1.0, 1.5, 2.0 IU/ml standard. Fit a straight line to the points by a minimum least square procedure. The tPA activity in the patient's plasma specimen can be determined by interpolation from the standard curve. Results from samples diluted 1:5 with Citrate Buffer should be multiplied by 5 for the tPA concentration.

Sample Calibration Curve

THE CALIBRATION CURVE BELOW IS A SAMPLE ONLY. Users must construct a standard curve each time the assay is performed.



X. QUALITY CONTROL

It is recommended that two mid-range plasma samples (0.25-1.50 IU/ml tPA), collected with a Biopool Stabilitye™ tube, are used as a quality control check each time the assay is performed. Do not use the tPA Activity Standard provided to construct the standard curve to check device performance.

XI. LIMITATIONS AND INTERFERENCES

1. Human fluids other than plasma have not been tested.
2. Plasma obtained with the usual citrate and EDTA blood collection tubes will typically give results which are abnormally low due to the presence of PAI-1, the fast-acting inhibitor of tPA, in blood⁽⁶⁾.
3. Abnormalities in tPA levels have been observed in the following conditions:

1. *Venous Thrombosis*: In patients with deep vein thrombosis, pulmonary embolism or superficial thrombophlebitis, a large percentage demonstrated abnormally low tPA activity following provocative testing^(1,3,4).
2. *Pregnancy*: tPA levels, as well as PAI levels, increase during pregnancy⁽¹²⁾.
3. *Post-operatively*: tPA abnormalities have been observed that may have value for prediction of deep vein thrombosis^(13,14).

XII. EXPECTED VALUES

The tPA activity in plasma varies. For healthy humans the basal level is between 0.2-2 IU/ml⁽¹¹⁾. tPA activities for healthy individuals following venous occlusion are reported to be 1.4-14 IU/ml⁽¹⁾.

XIII. PERFORMANCE CHARACTERISTICS

The user should establish product performance characteristics for the specific instrumentation used.

A. Precision

Within run and run to run precision was determined at two different levels of tPA.

Sample containing 1.3 IU/ml:	CV=3.9% (within run, n=20)
	CV=5.2% (run to run, n=10)
Sample containing 0.45 IU/ml:	CV=7.0% (within run, n=20)
	CV=5.3% (run to run, n=10)

B. Accuracy

The Chromolize™ tPA kit was compared to the Biopool Spectrolyse™ fibrin kit on 75 patient samples ranging from 0.02 to 2.75 IU/ml tPA. The correlation coefficient was 0.981; $y = 1.009x + 0.0526$.

C. Sensitivity

Assay sensitivity is ensured by acidification of the sample during blood draw, which neutralises plasminogen activator inhibitors as well as plasmin inhibitors. The standard curve is linear between 0 and 2.0 IU/ml. The assay is insensitive to plasminogen and fibrinogen^(6,15).

D. Specificity

Assay specificity is ensured by the stimulators poly-lysine and FDP. Under assay conditions, these stimulators increase the activity of tPA on plasminogen about 300-fold, but have no effect on urokinase⁽¹⁵⁾.

Specificity of the method is indicated by the ability of specific antibodies to tPA to quench activator activity⁽¹⁵⁾.

XIV. REFERENCES

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XV. KEY GUIDE TO SYMBOLS



Use by



Lot



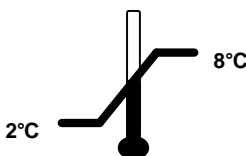
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For *in vitro* diagnostic use



Store at 2-8°C



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