



## **tPA CASSIA KIT**

### **Colorimetric Active Site-Specific Immunoassay**

***Catalog No. CK-101  
For Research Only***

#### **SUMMARY AND EXPLANATION**

An irreversible serine protease inhibitor labeled with a biotin moiety has been incorporated into the tPA CASSIA draw tubes. Therefore, the tPA CASSIA specifically measures only the plasma tissue-type plasminogen activator which has incorporated the biotin-labeled inhibitor, i.e. that which was active at the time of specimen collection. The capture antibody recognizes both single- and two-chain tPA. The conjugate employed is peroxidase-labeled avidin, and therefore detects only the biotin-labeled tPA bound to the plate. Upon the addition of substrate to the wells, color development is proportional to the concentration of biotin-labeled tPA.

#### **KIT INSTRUCTIONS**

**Reagents and Materials Supplied:** Sufficient reagents are supplied to assay 20 patient plasma samples, allowing 4 wells per patient.

- 1 antibody-coated immunoassay plate in remove-a-well format
- 20 x 2ml evacuated anticoagulant draw tubes
- tPA-X-biotin standard, 40ng
- extravidin peroxidase (XAP) conjugate (1000x), 40 $\mu$ l
- 20ml of 100x buffered-detergent solution
- 35g sodium chloride (NaCl)
- 500mg bovine serum albumin (BSA)
- 2x15mg o-phenylenediamine (OPD) tablets
- 20ml of 2x OPD substrate buffer

### Reagents and Materials Required but not Provided:

- glassware: 1 with 2L capacity and 1 with 500ml capacity
- distilled or deionized water
- dilution tubes: 1ml, 5ml, 20ml
- micropipets capable of delivering a range of volumes, 5µl-1000µl, and disposable tips
- one 8 or 12 channel pipet capable of delivering 50µl-150µl/channel and disposable tips
- reagent reservoirs
- microtiter plate washer or wash bottle
- blotting paper
- 4N H<sub>2</sub>SO<sub>4</sub>
- microtiter plate reader capable of reading absorbance values at 490nm

**Storage and stability:** Recommended storage temperature for all unused kit components is 4°C. Reagents are stable for a minimum of 6 months from the date of receipt if stored as recommended.

**\*This kit is intended for research only, and is not to be used for diagnostic purposes.**

### Buffer preparation:

1. Wash buffer: dilute 20ml of 100x buffered detergent solution to 2L with distilled water and add 35g NaCl. Stir to dissolve.
2. Assay buffer (AB): Reconstitute BSA with 5ml of wash buffer and then dilute with 495ml wash buffer. Stir well to dissolve.

### Method:

#### Specimen collection and storage:

1. **Transfer** 2ml of whole blood to tPA CASSIA tubes immediately after blood is drawn. Mix well by inversion. **Note:** Do not draw blood directly into evacuated anticoagulant draw tubes, as these tubes are provided non-sterile.
2. Centrifuge at 2000g for 15min. Remove plasma layer and store @ -20°C or less in working aliquots.

#### Assay procedure:

1. Reconstitute the tPA-X-biotin standard with 2ml of AB to 20ng/ml. Make 6 serial 1:2 dilutions in AB to 0.312ng/ml (400µl sample + 400µl assay buffer). The standard curve should be run in duplicate. If the standard is to be re-used, store the unused portion @ -20°C.
2. Thaw patient plasma samples at 37°C and maintain on ice. Dilute patient plasma samples accordingly in AB to fit within the standard curve range. The minimum recommended plasma dilution is 1:10; other recommended dilutions are 1:50 (10µl plasma + 490µl AB), 1:100 (5µl plasma + 495µl AB), 1:200 (5µl plasma + 995µl AB).
3. Using microtiter plate washer or wash bottle, wash wells of microtiter plate 3x with wash buffer and allow to drain well on blotting paper (without drying out) before continuing.
4. Add 150µl of sample or standard to each well of microtiter plate. Leave a minimum of 2 wells for the plate blank. To the blank wells, add 150µl of AB.
5. Allow plate to incubate for 1hr at room temperature (covered with plastic).
6. Wash wells 5x with wash buffer and allow plate to drain well.
7. Dilute XAP conjugate concentrate 1:10 by adding to it 0.36ml of assay buffer, yielding a 100x

conjugate solution. Mix by inversion. Calculate the required volume of working conjugate solution needed by multiplying (.15ml) x the # of wells being used. Then dilute the 100x conjugate solution to 1x with assay buffer to yield sufficient quantity of working conjugate solution. For an entire plate, make 16ml of working conjugate solution. Store the unused portion of the 100x solution @ 4°C.

8. Add 150µl of diluted XAP conjugate per well of microtiter plate, cover with plastic, and incubate 30min at room temperature.
9. Wash wells 5x with wash buffer, rinse with distilled water to remove bubbles, and allow plate to drain very well.
10. Dilute 8ml of 2x OPD substrate buffer with 8ml distilled water. Add 1x15mg OPD tablet and allow to dissolve.
11. Add 150µl of substrate solution per well.
12. When the highest concentration of standard has developed to the point of reading an absorbance of approximately 1-2 OD units (4-5 minutes), stop the reaction by the addition of 50µl/well of 4N H<sub>2</sub>SO<sub>4</sub>.
13. Read the absorbance at 490nm between 5-15 minutes after stopping the reaction. Suggested curve fit is 4-parameter. For greatest accuracy, select values for the unknowns that fall in the mid-range of the standard curve.

**For technical information, call 802-878-1777.**

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