

tPA assay kit

Mouse tPA activity ELISA Kit

CATALOG #
AAA71824

PRODUCT SYNONYMS

tPA; Mouse tPA activity ELISA Kit; Mouse tPA Activity Assay; tPA assay kit

PRODUCT SPECIFICATIONS

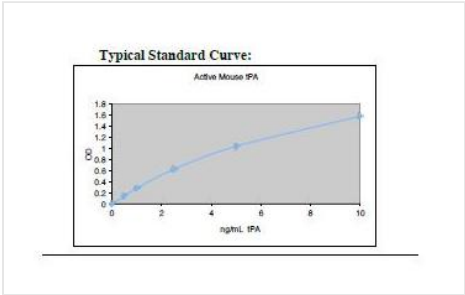
Sequence Length	19
Procedural Notes	1. This assay should be run at room temperature. 2. Use aseptic technique when opening and dispensing reagents. 3. This kit is designed to work properly as provided and instructed. Additions, deletions or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay. 4. To minimize error due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel.
Reagent Preparation	1. 10x Wash Buffer: Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use. 2. TBS Buffer: 0.1 M Tris, 0.15 M NaCl, pH 7. 4. 3. 3% BSA Blocking Buffer: 3% BSA in TBS Buffer. 4. Biotinylated PAI-1: Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use. 5. Standard: Reconstitute with 1.0 mL 3% BSA Blocking Buffer immediately prior to use and vortex gently to mix. The vial is now 1,000 ng/mL tPA. Prepare the standards according to the Dilution Table below. 6. Primary Antibody: Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use. 7. Secondary Antibody: Dilute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.

Standard Preparation	Standard tPA Concentration (ng/mL) Blocking Buffer (μL) Transfer Volume (μL) Transfer Source Final Volume (μL) S10 50 950 50 Stock Vial 500 S9 25 500 500 S10 600 S8 10 600 400 S9 500 S7 5 500 500 S8 600 S6 2 600 400 S7 500 S5 1 500 500 S6 500 S4 0.5 500 500 S5 500 S3 0.25 500 500 S4 600 S2 0.1 600 400 S3 500 S1 0.05 500 500 S2 1,000 S0 0 500 --- --- 500
Sample Collection, Storage and Preparation	Samples should be collected using 0.1 M trisodium citrate or acidified citrate in a 1:10 ratio of collection media to blood. Immediately upon collection of blood, the samples should be centrifuged at 3000 x g for 15 minutes. This should ensure the removal of platelets as they can release PAI-1, that in turn complexes with tPA. The plasma should be transferred to a clean plastic tube to be kept on ice prior to analysis or stored frozen for up to one month. Samples are stable for approximately 24 hours when stored at 4 degree C or one month if stored at -20 degree C and thawed three times without loss of tPA activity. Note: Detergents such as Triton X cause interference with the assay. If using detergent extracted samples, it is necessary to dialyze the samples overnight to remove the detergent.
Preparation and Storage	1. Store the kit and all of its components at 4 degree C before use. 2. If not using the entire plate at once, prepare only the appropriate amount of Primary Antibody and PAI-1 Standard. The remaining stock solutions should be frozen and stored at At -70C. Primary Antibody should be used within two weeks. All other components should remain refrigerated. 3. Store the reconstituted Biotinylated PAI-1 at 4 degree C. Use within two weeks. 4. Store unused portions of the microplate in a pouch with a desiccant at 4 degree C.

IMAGES

Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S0	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	U1
B	S0	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	U1
C	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13
D	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13
E	U14	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24	U25
F	U14	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24	U25
G	U26	U27	U28	U29	U30	U31	U32	U33	U34	U35	U36	U37
H	U26	U27	U28	U29	U30	U31	U32	U33	U34	U35	U36	U37



Application Data

Application Data

ADDITIONAL INFORMATION

Related Product Information for tPA assay kit	Introduction: Tissue-type Plasminogen Activator (tPA) is a member of the serine proteinase family. tPA functions to lyse fibrin clots into soluble plasmin fragments. tPA is active in two forms, single chain and two-chain. The two-chain tPA is created via interaction with the plasmin product cleaving the single chain. This two-chain form is regarded as the more active form. Both single chain and two-chain tPA are complexable with PAI-1. PAI-1 acts as an inhibitor for tPA by binding to the tPA and thus stifling its ability to lyse fibrin. tPA can serve as an indicator of both myocardial infarction for patients with impaired fibrinolytic systems as well as a marker for type-II diabetes. Principle of assay: This is an ELISA (Enzyme-Linked Immunosorbent Assay) for the quantitative analysis of active tPA levels in biological fluid. This test kit operates on the basis of sandwich ELISA where free active tPA enzyme complexes with PAI-1 and is quantitated with the use of an HRP labeled secondary antibody. First the biotinylated PAI-1 binds to the avidin coated wells. Next, active tPA present in the standard or unknown, complexes with PAI-1. Inactive or complexed tPA is removed in a subsequent wash step. A primary antibody specific for tPA is then added to each well followed by the HRP conjugated secondary antibody. The bound conjugated secondary antibody is detected by the addition of substrate, which generates an optimal color after 10 minutes. Quantitative test results may be obtained by the measure and comparison of the sample and standard absorbance readings when read with a microplate reader at 450 nm.
References	1. Thogersen A et al. (1998) Circulation 98: 2241-2247 2. Eliasson M et al. (2003) Cardiovascular Diabetology 2:19

NCBI AND UNIPROT INFORMATION

NCBI GI #	2285954
NCBI GeneID	5327
UniProt Accession #	Q6LBF5; P00750
Molecular Weight	2,342 Da
NCBI Official Full Name	tPA, partial
NCBI Official Synonym Full Names	plasminogen activator, tissue
NCBI Official Symbol	PLAT
NCBI Official Synonym Symbols	TPA; T-PA
NCBI Protein Information	tissue-type plasminogen activator; alteplase; plasminogen activator, tissue type; plasminogen/activator kringle; reteplase; t-plasminogen activator; tissue plasminogen activator (t-PA)
UniProt Protein Name	TPA protein
UniProt Gene Name	tPA
UniProt Entry Name	Q6LBF5_HUMAN

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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Online datasheet: <https://stage.aaabiotech.com/assay-kits/tpa/71824>