

tPA assay kit

Human tPA activity ELISA Kit

CATALOG #
AAA71822

PRODUCT SYNONYMS

tPA; Human tPA activity ELISA Kit; Human 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 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare according to included Standard Dilution Table immediately prior to use. 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	1. Reconstitute the standard by adding 10 mL of blocking buffer directly to the vial and agitate gently to completely dissolve the contents. The concentration will be 61 IU/mL. 2. Dilute 100 uL of standard into 900 uL of blocking buffer. The standard is now 6.1 IU/mL. 3. Use the dilution table below to prepare all standards for the assay. <table> <tr><td>tPA</td><td></td></tr> <tr><td>Concentration</td><td></td></tr> <tr><td>(IU/mL)</td><td></td></tr> <tr><td>uL of 6.1 IU/mL</td><td></td></tr> <tr><td>tPA Standard</td><td></td></tr> <tr><td>uL of Blocking</td><td></td></tr> <tr><td>Buffer</td><td></td></tr> <tr><td>Total Volume</td><td></td></tr> <tr><td>1 100 510 610</td><td></td></tr> <tr><td>.5 50 560 610</td><td></td></tr> <tr><td>0.4 40 570 610</td><td></td></tr> <tr><td>0.25 25 585 610</td><td></td></tr> <tr><td>0.1 10 600 610</td><td></td></tr> <tr><td>0.05 5 605 610</td><td></td></tr> <tr><td>0.02 2 608 610</td><td></td></tr> <tr><td>0.01 1 609 610</td><td></td></tr> <tr><td>0 0 500 500</td><td></td></tr> </table>	tPA		Concentration		(IU/mL)		uL of 6.1 IU/mL		tPA Standard		uL of Blocking		Buffer		Total Volume		1 100 510 610		.5 50 560 610		0.4 40 570 610		0.25 25 585 610		0.1 10 600 610		0.05 5 605 610		0.02 2 608 610		0.01 1 609 610		0 0 500 500	
tPA																																			
Concentration																																			
(IU/mL)																																			
uL of 6.1 IU/mL																																			
tPA Standard																																			
uL of Blocking																																			
Buffer																																			
Total Volume																																			
1 100 510 610																																			
.5 50 560 610																																			
0.4 40 570 610																																			
0.25 25 585 610																																			
0.1 10 600 610																																			
0.05 5 605 610																																			
0.02 2 608 610																																			
0.01 1 609 610																																			
0 0 500 500																																			

Sample Collection, Storage and Preparation

Samples of human plasma in citrate or EDTA may be assayed with this kit. Plasma in heparin is not recommended. It is important to ensure a platelet free preparation as platelets can release PAI-1, which in turn could potentially form a complex with active tPA. Serum and cell culture media at neutral pH may also be used. For best results, collect 9 volumes of blood in 1 volume of 0.1M acidified citrate, preferably using Stabilyte evacuated vials

5. The low pH of the resulting plasma insures that PAI- 1 is inhibited from quenching tPA activity

6. Immediately after collection of blood, samples must be centrifuges at 2500 x g for 15 minutes. The plasma must be transferred to a clean plastic tube and stored on ice prior to analysis. The tPA activity samples collected in the Stabilyte media are stable for up to 5 hours on ice, up to one month frozen at -20 degree C or up to 5 months frozen at -70 degree C.

Preparation and Storage

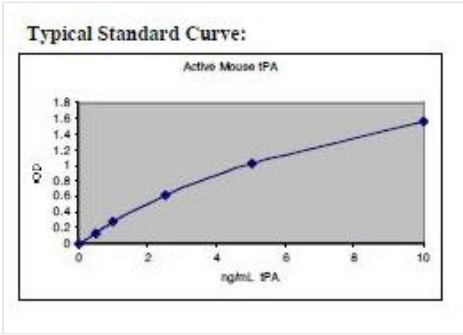
1. Store the kit and all of its components at 4 degree C before use.
2. If not using the entire plate at once, prepare only the appropriate amount of Primary Antibody and PAI-1 Standard. The remaining stock solutions should be frozen and stored at At -70C. Primary Antibody should be used within two weeks. 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	U1	U2
B	S0	S1	S2	S3	S4	S5	S6	S7	S8	S9	U1	U2
C	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13	U14
D	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13	U14
E	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24	U25	U26
F	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24	U25	U26
G	U27	U28	U29	U30	U31	U32	U33	U34	U35	U36	U37	U38
H	U27	U28	U29	U30	U31	U32	U33	U34	U35	U36	U37	U38

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 fragments¹. 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 twochain 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. Clinical studies have indicated that high tPA levels may increase the risk for thrombosis², whereas decreased levels may cause neuronal plasticity and degeneration³.

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. Sallés, Fernando J., et al.; (2002) J. Neuroscience 22(6):2125-2134 2. Wiman, Björn, et al.; (1984) J. Bio. Chem. 259(6):3644-3647 3. Hastings, Gregg A., et al.; (1997) J. Bio. Chem. 272(52):33062-33067 4. Yang, Y.H., et al.; (2001) J. Immunol. 167(2):1047-1052 5. Ranby, M., et al.; (1989) Thromb. Haemost. 62(3):917-922 6. Chmielewska, J., et al.; (1986) Clin. Chem. 32(3):482-485 7. Kruithof, E.K., et al.; (1987) Blood 69(2):460-466 8. Eliasson, et al.: (1993) Fibrinolysis 7:316-323
-------------------	---

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.

AAA Biotech | Tel: +1 (619) 604-9440 | Fax: +1 (619) 604-9441 | contact@aaabiotech.com
Online datasheet: <https://www.aaabiotech.com/assay-kits/tpa/71822>