SOP: Human tPA Activity ELISA Kit

Approvals

Preparer: Jason McMillan Date: 10APR14
Reviewer: Dr. Margaret Bryans Date: 11APR14

1. **Purpose**
   1.1. Quantitative determination of active tPA
2. **Scope and Applicability**
   2.1. Human tPA Total Antigen ELISA may be used for quantitative determination of total tPA in cell culture and tissue lysate samples as well as human plasma and other biological fluids.

3. **Summary of Method**
   3.1. Biotinylated Human PAI-1 Addition
   3.2. Preparation of Standard
   3.3. Standard and unknown addition
   3.4. Primary antibody addition
   3.5. Secondary antibody addition
   3.6. Substrate incubation
   3.7. Measurement
   3.8. Calculation of results
4. **References**
   4.1. Molecular Innovations Human tPA Activity ELISA Kit (Cat # HTPAKT) Manual
   4.2. Bio Rad iMark Microplate Absorbance Reader SOP
5. **Precautions**
   5.1. None
6. **Responsibilities**
   6.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   6.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
7. **Equipment and Materials**
   7.1. Molecular Innovations Human tPA Activity ELISA Kit (Cat # HTPAKT)
   7.2. 20µl, 200µl, and 1000µl pipettes and tips
   7.3. Shaking platform capable of reaching 300rpm
   7.4. Bio Rad iMark Microplate Absorbance Reader
   7.5. Microtubes and rack
   7.6. Blocking Buffer (3% BSA (w/v) in TBS buffer (0.1M Tris, 0.15M NaCl, pH 7.4))
   7.7. 1M HCl
   7.8. 1X washing Buffer
   7.9. tPA Samples from Spinner Flask and Bioreactor

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8. Procedure

8.1. Biotinylated Human PAI-1 Addition

8.1.1. Remove microtiter plate from the bag and add 100µl of Biotinylated Human PAI-1 to all wells.

8.1.2. Shake plate at 300rpm for 30 minutes.

8.1.3. Wash wells three times with 300µl of wash buffer. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or kimwipe.

8.2. Preparation of Standard (Dilutions for the standard curve and zero standard must be made and applied to the plate immediately)

8.2.1. Dilute 100µl of 61 IU/ml standard with 900µl blocking buffer to create a 6.1 IU/ml standard. Follow dilution table located in Attachments Section for standard preparation.

8.3. Add 100µl of tPA standards and unknowns to their individual wells. Be sure to carefully record their position on the microtiter plate.

8.3.1. Shake plate at 300rpm for 30 minutes.

8.3.2. Wash wells three times with 300µl of wash buffer. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or kimwipe.

8.4. Primary Antibody Addition

8.4.1. Add 100µl of primary antibody to all wells.

8.4.2. Shake plate at 300rpm for 30 minutes.

8.4.3. Wash wells three times with 300µl of wash buffer. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or kimwipe.

8.5. Secondary Antibody Addition

8.5.1. Add 100µl of primary antibody to all wells.

8.5.2. Shake plate at 300rpm for 30 minutes.

8.5.3. Wash wells three times with 300µl of wash buffer. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or kimwipe.

8.6. Substrate Incubation

8.6.1. Add 100µl of TMB substrate to all wells and shake at 300rpm for approximately 4 minutes. Substrate will change from colorless to different shades of blue.

8.6.2. Quench reaction by adding 50µl of 1M HCl in the same order as the substrate was added to stop the reaction when the sample shades of blue match the gradient of blue in the standards. The color will change from blue to yellow. Mix thoroughly by gently shaking the microtiter plate by hand for approximately one minute.

8.7. Measurement

8.7.1. Measure the absorbance in all wells at 450nm using the Bio Rad iMark Microplate Absorbance Reader.

8.8. Calculation of Results

8.8.1. Subtract the value of the zero point standard from all of the standards and unknowns to determine the corrected absorbance (A_{450}).

8.8.2. Plot A_{450} against the amount of active tPA in the standards to create a Full Range Active Human tPA in BSA standard curve.

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8.8.3. Fit a straight line through the linear points of the Full Range Active Human tPA in BSA standard curve to create a Linear Range Active Human tPA in BSA standard curve.

8.8.4. The amount of tPA in the unknowns can be determined from the standard curve.

8.8.5. Create a graph showing activity of tPA over time in days.

9. Attachments

tPA Concentration (IU/ml)	µl of 6.1 IU/ml tPA Standard	µl of blocking buffer	Total volume (µl)
1	100	510	610
0.5	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

Figure 1. Dilution table for preparation of human tPA standard

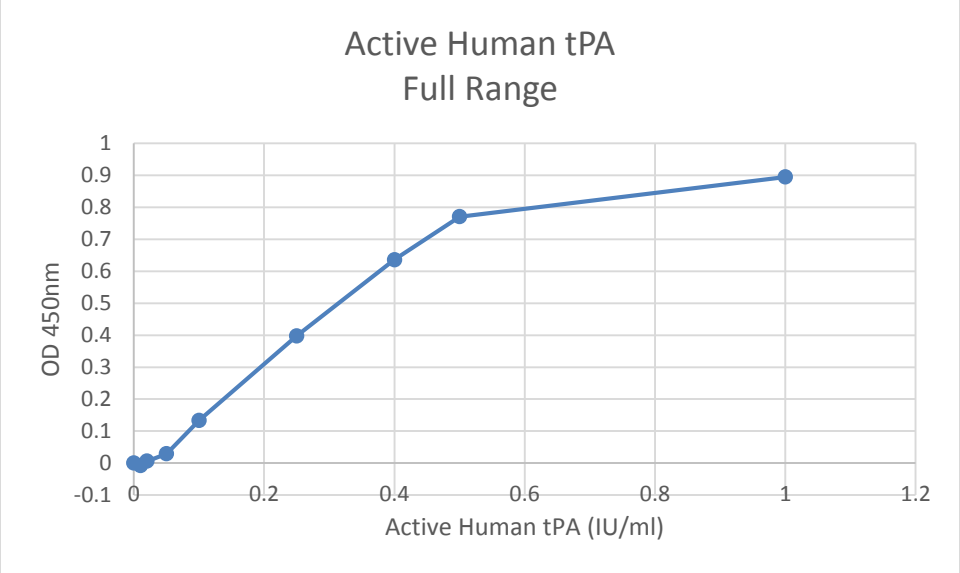

Figure 2. Active Human tPA Full Range (Example Only)

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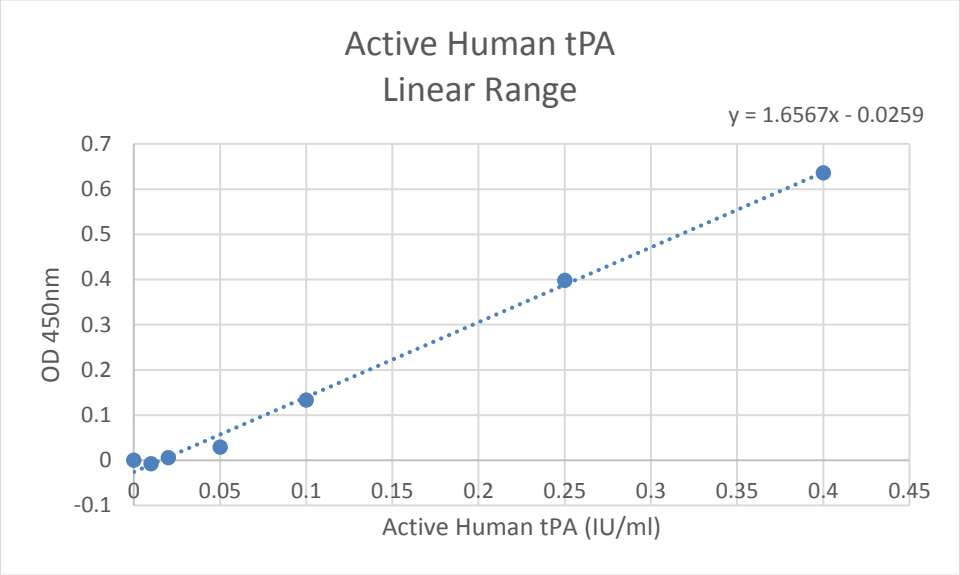

Figure 3. Active Human tPA Linear Range (Example Only)

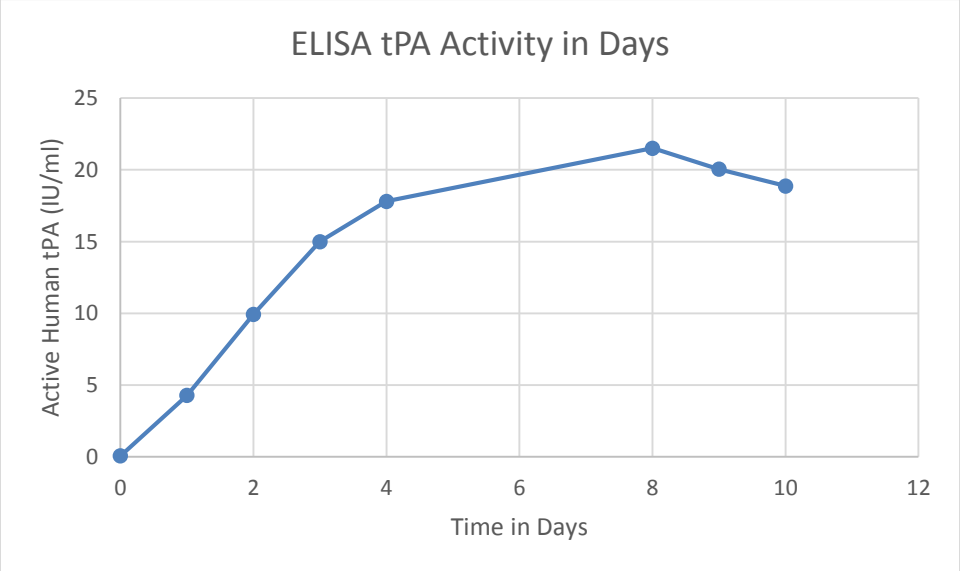

Figure 4. tPA Activity Over Time (Example Only)

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	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Figure 5. ELISA Plate Layout

10. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	09APR14	Jason McMillan	Initial release