Title: tPA Activity Assay SOP

Approvals:
Preparer:_____Deb Audino_____ Date:_____03Apr08_____
Reviewer:_____Bob O'Brien_____ Date: _____03Apr08_____

1. **Purpose:**
   1.1. To measure tPA activity.
2. **Scope:**
   2.1. To measure tPA activity from cultured cells or purified fractions using the Spectrozyme substrate.
3. **Responsibilities:**
   3.1. 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.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to informed the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. pH meter SOP
   4.2. incubator SOP
   4.3. plate reader SOP (optional)
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. pH meter
   7.2. 37ºC incubator
   7.3. plate reader with 450nm filter (optional)
   7.4. 250mL flask
   7.5. 100mL graduated cylinder
   7.6. 125mL bottle
   7.7. 1 and 10mL pipets and pump
   7.8. 10mL tube
   7.9. 1X Tris-Imidazole buffer with pH 8.4
      7.9.1. 4.04 g tris-base
      7.9.2. 2.27g imidazole
      7.9.3. 13.65g NaCl (sodium chloride)
   7.10. microtiter strips
   7.11. laboratory film such as Parafilm
   7.12. 10µM SPECTROZYME® substrate from American Diagnostica (Catalog number: 444)
   7.13. 10µL (0.04 µg/µL) tPA
   7.14. 1N HCl (or any hydrochloric acid solution for adjusting pH)
8. **Procedure:**
   8.1. **Solution Preparation**
      8.1.1. **10X Tris-Imidazole, pH 8.4.**
         8.1.1.1. Weigh and combine the following chemicals in a 250mL flask:
            4.04g tris-base

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2.27g imidazole
13.65g NaCl (sodium chloride)

8.1.1.2. Add 94mL deionized water.
8.1.1.3. Add 1N HCl (Hydrochloric acid) drop-wise until pH of solution is 8.4.
Note: 1N HCl is recommended, but it is acceptable to use any hydrochloric acid solution approved by the instructor for pH adjustment.
8.1.1.4. Transfer to a 100mL graduated cylinder.
8.1.1.5. Bring volume up to 100mL with deionized water.
8.1.1.6. Transfer to a 125mL bottle.
8.1.1.7. Store at room temperature.

8.1.2. **1X Tris-Imidazole , pH 8.4**
8.1.2.1. Using 1mL and 10mL pipets, combine 1mL of 10X Tris-Imidazole, pH 8.4 and 9mL distilled water in a 10mL tube.
8.1.2.2. Store at room temperature.

8.1.3. **Spectrozyme (5nmol/mL)**
8.1.3.1. Add 2mL deionized water to lyolphilized 10 µMoles SPECTROZYME®.
8.1.3.2. Be sure that the powder is fully dissolved by inverting the bottle several times.
8.1.3.3. Storage: Reconstituted substrate may be stored for 1 week at room temperature, 2 months at 2-8ºC, or up to 6 months at –20ºC (Aliquot and freeze. Do not submit to freeze-thaw cycles).

8.2. **Assay**
8.2.1. Add 80 µL 1X Tris-Imidazole to each well
8.2.2. Add 20µL of 5nmol/mL SPECTROZYME® substrate to each well.
8.2.3. Prepare control wells.
8.2.3.1. Positive (+) Control Well: add 10 µL tPA (0.04 µg/µL) into positive (+) well.
8.2.3.2. Negative (-1) Control Well: add 10 µL Tris-Imidazole buffer into negative (-1) well.
8.2.3.3. Negative (-2) Control Well (only if testing a culture sample): add 10µl media without cells into negative (-2) well.
8.2.4. Prepare sample wells.
8.2.4.1. Place 10µL of each sample into their specified well.
8.2.5. Gently shake microtiter strip well to mix reagents.
8.2.6. Cover the strip with laboratory film.
8.2.7. Incubate wells in 37°C incubator for 1-24 hours.
8.2.8. Remove from incubator and observe tubes. Positive tubes should turn yellow while negative tubes should stay clear.
8.2.9. Optional: Read wells in a plate reader set at 450nm.

9. Attachments: N/A

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10. History:

Name	Date	Amendment
Christopher Cotter Amanda Marshall	01 Jul04	Initial release
Deb Audino	01Jun05	Put into 2005 SOP format and reduced volume of assay.
Deb Audino	04Nov05	Added catalog number of Spectrozyme. Edited adding reagents to wells to clarify the section
Deb Audino	04Apr08	Updated history format. College name change