

# Determination of Urokinase Activity

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## Method Article

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# Abstract

An important step in the protocol: “Disposable Polymeric Cryogel Bioreactor Matrix for Therapeutic Protein Production”, is testing the circulating media at regular time intervals to analyze for glucose, lactic acid, and the protein of interest being secreted by the cells. In this Exchange Protocol we describe our method for determining the concentration of urokinase, an example protein of interest. Urokinase activity was determined as amidolytic effect on the substrate pyro-Glu-Gly-Arg-p-nitroanilide-HCl. The rate of formation of p-nitroaniline was measure at 405nm using acid stopped method. Urokinase activity vs absorbance correlation is linear between 5-40 Ploug units.

## Reagents

pyro-Glu-Gly-Arg-p-nitroanilide-HCl \ (S2444 Chromogenix Art. No. 82 03 57 Lexington USA) Reconstitute the substrate S-2444 \ (MW: 498.9) with 16.7 ml of distilled water \ !CAUTION Harmful by inhalation Urokinase from human kidney cells \ (activity 0.37 Sigma units/mg protein) Sigma U4010 \ (Reconstitute with sterile 0.2ml water) 20% Acetic acid Tris Buffer, pH 8.8 \ (25°C) containing Tris \ (50 mmol/l), NaCl \ (38 mmol/l). Adjust pH to 8.8 by adding required amount of \ (about 12ml) 1mol/l HCL. The buffer is stable at 2-8°C for two months. Note: To avoid risk of other proteases add aprotinin \ (10 KIU/ml) in case buffer or urokinase solution is contaminated.

## Procedure

1. Prepare urokinase standard by dilution of urokinase stock solution to 400 units/ml using water. Dilute urokinase solution \ (400 units/ml) in Tris buffer to 300, 200, 100. 50 units. 2. Add 200µl of Tris buffer to each well in a 96 well plate and incubate at 37°C for 5min. 3. Add 25µl of standard and sample \ (media sample from reactor, and eluate post purification). 4. Incubate at 37°C for 1-2min 5. Add 25µl of substrate to each well and incubate at 37 C for 5 min. 6. Add 25µl of 20% acetic acid to stop the reaction. Incubate for 5 min 7. Simultaneously prepare a solution, following the same procedure, by adding 25µl of Tris buffer instead of standard or sample urokinase. 8. Mix the solution well and measure absorbance at 405 nm. The color is table for 4h. 9. Calculate the urokinase activity as  $U/I = A \times 229$  \ (where A is absorbance)

## Timing

Step 1-9: 30-45 min