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Colorimetric determination of urea V.2

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ABSTRACT

This protocol measures the absorbance of urea in solution in complexation with diacetyl monoxomie at 520 nm and is linearly proportional to concentration up to 5 mM urea.

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MATERIALS TEXT

thiosemicarbazide, diacetyl monoxime, water, sulfuric acid, phosphoric acid, ferric chloride

SAFETY WARNINGS

This protocol utilizes strong acids. Ensure proper precautions and safety equipment are used when dealing with these chemicals.

BEFORE STARTING

Ensure a lab coat, lab goggles, and plastic gloves are worn throughout this assay.

Mixed Acid Reagent Preparation

1

Dissolve **2.5 mg** ferric chloride in **45 mL** deionized water in a 250 mL volumetric flask.

2 Add **80 µl** phosphoric acid.

3

> Caution: Preparation of **18 Molarity (M)** sulfuric acid is highly exothermic. Slowly add acid to **100 mL** water and stir frequently to avoid spattering.

Prepare **18 Molarity (M)** sulfuric acid by diluting **65.25 mL** concentrated sulfuric acid up to **250 mL** with deionized water.

4 Dilute ferric chloride, deionized water, and phosphoric acid mixture to **250 mL** with **18 Molarity (M)** sulfuric acid.

5 Mix until dissolved.

Mixed Color Reagent Preparation

6 Add **20.9 mg** diacetyl monoxime and **52.4 mg** thiosemicarbazide to a 250 mL volumetric flask.

7 Dilute to volume (**250 mL**) with deionized water.

8 Mix until dissolved.

Urea Assay 10m 5s

9 Prepare known concentrations of urea at **0 Milimolar (mM)** , **1 Milimolar (mM)** , **2 Milimolar (mM)** , **3 Milimolar (mM)** , **4 Milimolar (mM)** , and **5 Milimolar (mM)** for a calibration curve.

10 Fill a 600 mL beaker with **200 mL** deionized water.

11 Place the 600 mL beaker on hot plate and bring to a boil.

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12 To test a sample, aliquot **1 mL** into a 20 mL glass test tube.

13 Add **2 mL** Mixed Acid Reagent.

14 Add **2 mL** Mixed Color Reagent.

15 Ensure contents of test tube are vigorously mixed by vortexing for at least **00:00:05** . 5s

16 Repeat steps 12-15 for each sample and standard to be analyzed.

17 Ensure test tubes are labeled and place in boiling water bath for **00:20:00** .

18 Remove tubes from water bath and let cool for **00:10:00** . 10m

19 Fill plastic cuvette to line with cooled solution.

20 Place sample cuvette in spectrophotometer and record absorbance at 520 nm. Blank against the standard solution with both reagents, but no urea.

21 Use the calibration curve to determine urea concentrations of unknown solutions.

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