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Colorimetric determination of urea

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1 Works for me dx.doi.org/10.17504/protocols.io.bsz3nf8n

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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 4.6 mM urea.

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KEYWORDS

urea, urea assay, colorimetric urea, urea test, thiosemicarbazide, diacetyl monoxime

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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.

Mixed Acid Reagent Preparation

1

Dissolve **2.5 mg** ferric chloride in **45 mL** DI H_20 in a 250 mL volumetric flask.

2 Add **80 µl** phosphoric acid.

3

> Caution: Preparation of **18 Molarity (M)** H_2SO_4 is highly exothermic. Slowly add acid to **100 mL** water and stir frequently to avoid spattering.

Prepare **18 Molarity (M)** H_2SO_4 by diluting **65.25 mL** concentrated H_2SO_4 up to **250 mL** with DI H_2O.

4 Dilute ferric chloride, DI water, and phosphoric acid mixture to **250 mL** with **18 Molarity (M)** H_2SO_4.

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 DI H_2O.

8 Mix until dissolved.

Urea Assay

9 Fill a 600 mL beaker with **200 mL** DI H_2O.

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

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

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

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

14 Mix by inverting test tube at least 3 times.

15 To prepare blank, repeat steps 11-14 using **1 mL** of DI H_2O instead of the sample.

16 Label test tubes and place in boiling water bath for **00:20:00**

17 Remove tubes from water bath and let cool to room temperature.

18 Fill plastic cuvette to line with cooled solution.

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

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