**UREA-TEST®**

**UREA 450**

Cat. No. 10003079

Store at (+2 to +8) °C

Reagent Set for the preparation of 1000 ml of working solutions used for the determination of urea in biological material. The Set is sufficient for at least 450 analyses.

**Principle**

Urea reacts with diacetylmonoxime in strongly acidic medium in the presence of thiosemicarbazide and Fe(III) ions to form a red coloured complex.

**References**


Chromý, V., Medek, J., Partl, V.: Čs. author’s certificate 157989

**Reagents**

1. Standard solution (10 ml)
   - urea 16.65 mmol/l

2. Diacetylmonoxime (10 tablets)
   - diacetylmonoxime 0.5 mmol,
   - thiosemicarbazide 0.08 mmol,
   - Fe(III)-Na EDTA 2.5 µmol/tablet

**Composition of reaction mixture**

- Diacetylmonoxime: 5.0 mmol/l
- Thiosemicarbazide: 0.9 mmol/l
- Sulfuric acid: 0.9 mol/l
- Fe(III)-Na EDTA: 25.0 µmol/l
- Volume ratio of serum/reaction mixture: 1/201

**Reference values**

- fS Urea (mmol/l): 2.5–8.3
- dU Urea (mmol/24 hrs): 333–583

The range of reference values is only approximate, it is recommended to all laboratories to verify the extension of reference interval for their concrete examined population.

**Reproducibility**

Approx. ±5 %

**Calibration and quality control**

BIO-LA-TEST® SOLUNORM Urea, Cat. No. 10003175

BIO-LA-TEST® SOLUNORM Glucose-Urea, Cat. No. 10003178

BIO-LA-TEST® LYONORM U, Cat. No. 10003176

BIO-LA-TEST® LYONORM P, Cat. No. 10003172

**Auxiliary reagents**

- (not included in the Set)

- Sulfuric acid solution, 1.8 mol/l
- Prepare by dilution of 50 ml of concentrated sulfuric acid, p.a. with distilled water in 500 ml volumetric flask.

- Trichloroacetic acid solution, 5 %
- Prepare by dilution of 5 g of trichloroacetic acid in 100 ml of distilled water.

**Working solutions**

**Solution 1**

In 50 ml volumetric flask dissolve 1 tablet of Reagent 2 in approx. 30 ml of distilled water at elevated temperature.

When cooled refill up to the mark with distilled water.

The solution may contain small insoluble residue.

Stability: several weeks when stored at (+15 to +25) °C.

**Solution 2**

Mix Solution 1 with sulfuric acid solution in the ratio 1:1.

Stability: 1 day when stored at (+15 to +25) °C.

**Procedure**

**Wavelength**

(490–540) nm

**Cuvette**

1 cm

**Temperature**

(+15 to +25) °C

In three thin-walled test tubes mix Solution 2 in the ratio 200:1 with serum, or with diluted urine (sample), or with Reagent 1 (standard), or with distilled water (control solution) and stir (e.g. 2.0 ml of Solution 2 and 0.01 ml of serum, or diluted urine, or distilled water, or Reagent 1, or distilled water).

Cover the test tubes with aluminium foil and place them for exactly 10 min into a boiling water bath. Then cool quickly in running tap water and read the absorbances of sample (A₁) and standard (A₂) against control solution. The reading must be completed within 15 min after cooling the test tubes.

Dilute urine sample with distilled water 1+50, or 1+100 prior to analysis (result x dilution).

<table>
<thead>
<tr>
<th>Pipette (ml)</th>
<th>Sample</th>
<th>Standard</th>
<th>Control solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>–</td>
<td>0.01</td>
<td>–</td>
</tr>
<tr>
<td>0.01</td>
<td>–</td>
<td>–</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Mix and place test tubes for exactly 10 min into boiling water bath, then cool in tap water and within 15 min read the absorbances of sample (A₁) and standard (A₂) against control solution.

**Calculation**

Urea (mmol/l) = 16.65 × \( \frac{A_2}{A_1} \)

**Notes**

If the urea concentration exceeds 23 mmol/l, dilute the sample with distilled water and repeat the analysis (result x dilution). For the determination of urea in hemolytic or lipemic sera it is necessary to deproteinize the sample prior to use with for example 5 % solution of trichloroacetic acid (mix in a test tube 0.10 ml of a sample with 1.00 ml of trichloroacetic acid solution and centrifuge. Using the same procedure dilute the standard urea solution). The next procedure is the same as for the analysis without deproteinization. This is applicable for blood as well.

The upper level of urea in serum depends on the protein intake in food. With the protein intake over 2.5 g/kg of body-weight per day, the upper level of normal urea values can be increased up to 10 mmol/l.

**Health protection**

For in vitro diagnostic use. To be handled by entitled and professionally educated person.

Reagent 2 contains in low concentration (2.7 %) very toxic thiosemicarbazide.

At work, unconditionally observe the rules of personal hygiene. Do not eat, drink or smoke. Use means of personal protection.

R25 Toxic if swallowed.

S22 Do not breathe dust.

S37 Wear suitable gloves.

S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

**First aid**

At an accidental ingestion drink up approx. 0.5 l of water. At an eye contact flush immediately and thoroughly with large quantity of water. At a skin contact wash the skin with soap and warm water. In all serious cases of health damage consult the physician.

**Waste disposal**

All tested samples should be treated as potentially infectious and with the contingent rest of the reagents should be liquidated in accordance with any other local and national regulations relating to the safe handling of such materials.

Put packaging paper waste and rinsed containers to recycling.

Date of last revision: 21. 1. 2011

Produced Erba Lachema s.r.o.

Karásek 1d 621 33 Brno, CZ

e-mail: diagnostics@lachema.com, www.lachema.com