

# Uric Acid Reagent

## Trinder

### PRODUCT SUMMARY

Stability	:	3 Months at 2-8°C
Linear Range	:	0.01 - 1.5 mmol/L (0.17 - 25 mg/dL)
Specimen Type	:	Serum, plasma or urine
Method	:	Endpoint
Reagent Preparation	:	Add specified volume of distilled or deionised water.

**IVD**

#### INTENDED USE

This reagent is intended for the in vitro quantitative determination of Uric Acid in human serum, plasma or urine.

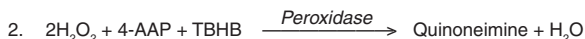
#### CLINICAL SIGNIFICANCE

Uric acid is a metabolite of purines, nucleic acids and nucleoproteins and abnormal levels may be indicative of a disorder in the metabolism of these substances. Hyperuricaemia may be observed in renal dysfunction, gout, leukemia, polycythaemia, atherosclerosis, diabetes, hypothyroidism, or in some genetic diseases. Decreased levels are present in patients with Wilson's Disease.<sup>1,2,3</sup>

#### METHODOLOGY

This reagent is based upon the methods of Trivedi and Kabasakalian<sup>4,5</sup> with a modified Trinder<sup>6</sup> peroxide assay using 2,4,6-Tribromo-3-hydroxy benzoic acid (TBHB).

The series of reactions involved in the assay system is as follows:



1. Uric Acid is oxidised to allantoin by uricase with the production of H<sub>2</sub>O<sub>2</sub>.
2. The peroxide reacts with 4-aminoantipyrine (4-AAP) and TBHB in the presence of peroxidase to yield a quinoneimine dye. The resulting change in absorbance at 520nm (520-550nm) is proportional to uric acid concentration in the sample.

#### REAGENT COMPOSITION

Active Ingredients	Concentration
4-Aminoantipyrine	0.35 mmol/L
TBHB	1.23 mmol/L
Uricase (Bacillus Sp.)	> 50 U/L
Peroxidase (Horseradish)	> 200 U/L
Tris Buffer	35 mmol/L

pH 8.25 ± 0.1 at 20°C.

**WARNING:** Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains Sodium Azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Uric Acid Reagent Material Safety Data Sheet. **The Packaging of This Product Contains Dry Natural Rubber.** Exercise precaution when handling crimps and broken glass vials, as sharp edges can injure the user.

- R25 Toxic if swallowed.  
 R32 Contact with acids liberates very toxic gas.  
 R36/38 Irritating to eyes and skin.  
 S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

#### REAGENT PREPARATION

Reconstitute the contents of each vial with the volume of distilled or deionised water stated on the vial label. Mix gently until dissolved.

#### STABILITY AND STORAGE

##### Prior to use:

When stored between 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

### SYMBOLS IN PRODUCT LABELLING

<b>EC REP</b>	Authorised Representative		Temperature Limitation
<b>IVD</b>	For in vitro diagnostic use		Use by/Expiration Date
<b>LOT</b>	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
<b>REF</b>	Catalogue number		Manufactured by
	Consult instructions for use		T - Toxic

#### Reconstituted Reagent:

When stored capped at 2-8°C, the reagent is stable for at least 3 months.

#### Indications of Reagent Deterioration:

- Turbidity;
- Reagent Absorbance > 0.5 AU at 520nm; and/or
- Failure to obtain control values within the assigned range.

#### SPECIMEN COLLECTION AND HANDLING

**Serum:** Use non - haemolysed serum.

**Plasma:** Use heparin or EDTA.

**Urine:** It is recommended that urine for uric acid determination be collected in 15 mL of 2 mol/L NaOH. Upon receipt, the pH should be checked. If the pH is < 8.0, it should be adjusted accordingly with NaOH to fall within the pH range of 8.0 to 8.5.<sup>7</sup>

**Storage:** Serum and plasma samples may be stored for at least 3 days at room temperature (18-25°C) and for at least 6 months frozen<sup>2</sup>. Urine samples when stored at room temperature are stable for 5 days.

#### ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance between 500 - 550 nm.
- Analyser specific consumables, eg: samples cups
- Distilled or deionised water for reagent preparation and related equipment eg: pipettes.
- Normal and Abnormal assayed controls.
- A suitable serum based calibrator or aqueous standard (see Calibration section).

#### ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

#### SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	520 nm (500-550nm)
Secondary Wavelength	660 nm (600-660nm)
Assay Type	Endpoint
Direction	Increase
Sample : Reagent Ratio	1:40 (1:40 - 1:50)
eg: Sample Vol	5 µL
Reagent Vol	200 µL
Incubation Time	240 seconds
Reagent Blank Limits (520nm, 1cm lightpath)	Low 0.0 AU High 0.5 AU
Linearity	0.01 - 1.5 mmol/L (0.17 - 25 mg/dL)
Sensitivity (520nm, 1cm lightpath)	0.42 ΔA per mmol/L (0.025 ΔA per mg/dL)

#### CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

$$\text{Uric Acid} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

#### Example:

Absorbance of calibrator	= 0.297
Absorbance of unknown	= 0.200
Value of calibrator	= 0.41 mmol/L (6.9 mg/dL)

$$\text{Uric Acid} = \frac{0.200}{0.297} \times 0.41 = 0.28 \text{ mmol/L}$$

$$\text{Uric Acid} = \frac{0.200}{0.297} \times 6.9 = 4.65 \text{ mg/dL}$$

#### NOTES

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- The colour development is stable for 15 minutes.
- Specimens with Uric Acid concentrations greater than 1.5 mmol/L (25 mg/dL) should be diluted with saline and reassayed. Multiply results by the dilution factor.
- Unit conversion factor: mmol/L x 16.8 = mg/dL.

#### CALIBRATION

Calibration is required. An aqueous standard or serum based calibrator, with an assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. Standards should not contain formaldehyde or enzyme inhibitors as preservatives. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications.

However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The lot number of reagent changes
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

#### QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling above the upper limit or below the lower limit of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

#### LIMITATIONS

- Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out and the following results were obtained:  
**Haemoglobin:** No interference from haemoglobin up to 160 mg/dL.  
**Free Bilirubin:** No interference from free bilirubin up to 855 µmol/L (50 mg/dL).  
**Conjugated Bilirubin:** No interference from conjugated bilirubin up to 103 µmol/L (6 mg/dL).  
**Lipaemia:** No interference from lipaemia, measured as triglycerides, up to 11.4 mmol/L (1000 mg/dL).  
**Ascorbic Acid:** No interference from ascorbic acid up to 0.9 mg/dL.
- Young DS<sup>®</sup> has published a comprehensive list of drugs and substances which may interfere with this assay.

#### EXPECTED VALUES<sup>9</sup>

Child:	0.12 - 0.33 mmol/L	2.0 - 5.5 mg/dL
Adult Male:	0.21 - 0.43 mmol/L	3.5 - 7.2 mg/dL
Adult Female:	0.15 - 0.36 mmol/L	2.6 - 6.0 mg/dL
Urine:	14.9 - 44.6 mmol/day	250-750 mg/day

The quoted values should serve as a guide only. It is recommended that each Laboratory verify this range or derives a reference interval for the population that it serves.

#### PERFORMANCE DATA

The following data was obtained using Uric Acid reagent on an automated clinical chemistry analyser.

#### IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure<sup>10</sup>.

Within Run:	LEVEL I	LEVEL II
Number of Data Points	80	80
Mean (mmol/L)	0.31	0.53
Mean (mg/dL)	5.26	9.02
SD (mmol/L)	0.005	0.006
SD (mg/dL)	0.08	0.10
CV%	1.6	1.1

Total:	LEVEL I	LEVEL II
Number of Data Points	80	80
Mean (mmol/L)	0.31	0.53
Mean (mg/dL)	5.26	9.02
SD (mmol/L)	0.008	0.011
SD (mg/dL)	0.13	0.19
CV%	2.5	2.1

#### ACCURACY

Comparison studies were carried out using another commercially available Uric Acid reagent as a reference. Serum and plasma (Heparin) samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	39
Range of sample results	0.06-0.89 mmol/L (0.99-14.88 mg/dL)
Mean of reference method results	0.60 mmol/L (10.1 mg/dL)
Mean of Uric Acid results	0.59 mmol/L (9.9 mg/dL)
Slope	1.008
Intercept	-0.015 mmol/L (-0.25 mg/dL)
Correlation coefficient	0.999

#### LINEARITY

When run as recommended, the assay is linear between 0.01 and 1.50 mmol/L (0.17 and 25 mg/dL).

#### SENSITIVITY

When run as recommended the sensitivity of this assay is 0.42 ΔA/min per mmol/L (0.025 ΔA/min per mg/dL).

#### REFERENCES

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REF

#### Reorder Information

Catalogue No.	Configuration
TR24010	20 x 10 mL
TR24015	20 x 20 mL
TR24003	10 x 50 mL
TR24004	10 x 200 mL
7500-025A	4 x 500 mL