

OXALATE

TRINDER COLORIMETRIC DETERMINATION IN URINE
 For in vitro diagnostic use only

Kit: 1 x 20 mL

Cod. OX8850

SUMMARY

Oxalate in urine is of elevated interest because it could originate a sparingly soluble calcium oxalate; this one could give calcium oxalate crystalluria and the stone formation in the urinary tract, who is considered the most important factor in urolithiasis.

Oxalate in urine may increase as an end-product of intermediary metabolism or from dietary sources.

A decreased excretion in the urine is associated to hyperglycinemia and hyperglycinuria.

An increased excretion could be due to increased ingestion of oxalate rich food or oxalate precursors; an increased one could be also due to metabolic defects or to the absorption of oxalate in several gastrointestinal disorders who give important fat malabsorption.

PRINCIPLE

Oxalate is oxidized to carbon dioxide and hydrogen peroxide by a very specific enzyme, oxalate oxidase.

Hydrogen peroxide reacts in presence of peroxidase (POD) with MBTH (3-methyl-2-benzothiazolinone hydrazone) and DMAB (3-dimethylamino benzoic acid) forming a blue quinone compound. The intensity of colour is proportional to the concentration of OXALATE in the sample and it is read at 590 nm.

REAGENTS

Components of the kit: **Cod. OX8850**

*REAGENT 1 (Buffer, liquid, READY TO USE)	1 x 20 mL
*REAGENT 2 (Iyo, to dilute ONLY WITH WATER)	1 x 2 mL
*REAGENT 3 (SAMPLE PURIFIER, powder) (READY TO USE)	20 tubes
*REAGENT 4 (SAMPLE DILUENT, liquid, 5x conc.) (to dilute with WATER to 100 mL)	1 x 20 mL
*REAGENT 5 Standard (liquid, READY TO USE) Oxalic acid (4,5 mg/dL = 45 mg/L = 0,5 mmol/L)	1 x 5 mL

Buffer pH 3,1 ± 0,1	> 20 mmol/L
Oxalate Oxidase (Barley)	> 2 KU/L
POD	> 1000 U/L
MBTH	> 0.2 mmol/L
DMAB	> 0.9 mmol/L

Activators, Stabilizers

STABILITY: the reagents, at 2-8°C, are stable up to the expiry date shown on the package **if not contaminated during handling.**

AUXILIARY REAGENTS FOR CURVE CALIBRATION

In order to have a Calibration Curve, we suggest the use of the following kit:

- **OXALATE Standard Set 3 x 50 mL**
Cod. OXST8853 (see the related insert)

AUXILIARY REAGENTS FOR QUALITY CONTROL

The reliability of test results should be monitored by routine use of artificial urine Controls of known concentrations. We suggest following kits:

- **OXALATE LOW CONTROL kit 6 x 2 mL**
Cod. OCL6627 (see the related insert)

- **OXALATE HIGH CONTROL kit 6 x 2 mL**
Cod. OCH6502 (see the related insert)

PREPARATION *REAGENT 1 (Buffer), *REAGENT 3 (Sample Purifier) and *REAGENT 5 (Oxalate Standard)

REAGENTS READY TO USE.

PREPARATION *REAGENT 2

***KIT 1 x 20 mL (Cod. OX8850)**

Add 2 mL of DISTILLED WATER to *Reagent 2. Mix gently until dissolution. **STABILITY:** 30 days at 2-8°C.

PREPARATION *REAGENT 4 (Sample Diluent)

***KIT 1 x 20 mL (Cod. OX8850)**

Add 80 mL of DISTILLED WATER to *Reagent 4. Mix gently until complete mixing. **STABILITY:** 3 months at 2-8°C.

Let the reagents reach the working temperature before use. Mix kindly before use. Close immediately after handling.

The Reagents have to be used properly, to avoid contamination. Incompetent handling will keep us harmless from any responsibility.

SAMPLE

• No haemolyzed fresh urine, with amount of vitamin C lower than 16 mmol/L, concentration who may affect the test results.

It is recommended that patients refrain from taking excessive amounts of vitamin C or vitamin C-rich-food for at least 48-72 hours prior the urine collection.

SAMPLE COLLECTION

A 24-hour urine sample is collected in a glass or plastic bottle containing 10 mL of conc. Hydrochloric acid.

Record the volume in litres.

Oxalate in acidified urine is stable for **7 days** when stored refrigerated or frozen.

SAMPLE PREPARATION

1. Prepare *REAGENT 4 (Sample Diluent) as mentioned before.
2. Set up a series of labelled tubes for urine samples and Controls.
3. Pipet 5 mL (or any available volume) of urine samples and Controls into proper labelled tubes.
4. Add 5 mL (or the same volume used in point 3.) of diluted SAMPLE DILUENT (*REAGENT 4) into each previous tubes and mix.
5. Check the pH: it have to be between 5.0 and 7.0. If not, adjust the pH with HCl 1N or NaOH 1N.
6. Set up a series of SAMPLE PURIFIER tubes (*REAGENT 3) for urine Samples and Controls.
7. Pipet 4 mL each of DILUTED urine Sample and Controls (see point 4. and 5.) to proper labelled SAMPLE PURIFIER tubes (see point 6.) and mix for about 5 minutes by intermittent mixing. We suggest a rotator mixer for mixing.
8. Centrifuge the tubes for 5 minutes at 3500 rpm (2600 x g) or filter on laboratory filter paper.
9. Determine the Oxalate concentration in the supernatants.
10. The Oxalate Standard (*REAGENT 5) DOES NOT REQUIRE the SAMPLE PREPARATION procedure; it is ready-to-use in the following ANALYTICAL PROCEDURE.

ANALYTICAL PROCEDURE

• Wavelength:	590 nm (570-620 nm)
• Pathlength:	1 cm
• Reading:	against air or dist. water
• Temperature:	37°C
• Method:	end-point
• Reaction:	5 minutes
• Linearity:	up to 2 mmol/L
• Sample/Reagent:	1/20/2

Let reagents reach the working temperature before using.



Pipette in 3 test tubes or cuvettes so labelled :

R/B: Blank Reagent, S: Sample and/or Controls, ST: Standard

	R/B	ST	S + Control
*Reagent 1 Buffer	2000 µl	2000 µl	2000 µl
Distilled water	100 µl	----	----
*Reagent 5 Standard DO NOT DILUTE	----	100 µl	----
Sample and Control diluted as in Sample Preparation	----	----	100 µl

Mix kindly and incubate at 37°C. After this one, add:

*Reagent 2	200 µl	200 µl	200 µl
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Mix carefully and incubate at 37°C for 5 minutes, waiting the end of the reaction. Read the absorbance of the standard (Ast) and of the sample (As) against the Reagent Blank.
The color is stable for at least 60 minutes.

CALCULATION

Oxalate in mmol/L =

$$\frac{[As / Ast] \times 0,50 \text{ (Standard conc.)} \times 2 \text{ (dilution factor)}}{\text{or}}$$

Plot each value found on the Calibration Curve (Cod. OXST8853).
The Calibration Curve has to be always repeated for each new lot of Reagent.

Oxalate excreted during 24-hour in mmol/24 h =

$$\text{Oxalate in mmol/L} \times \text{Volume of Urine voided in 24-h (in liters)}$$

Oxalate excreted during 24-hour in mg/24 h =

$$\text{Oxalate in mmol/24 h} \times 90 \text{ (MW)}$$

REFERENCE VALUES

Adults Males	7 - 44 mg/24h	(0,08 – 0,49 mmol/24h)
Adults Females	4 - 31 mg/24h	(0,04 – 0,32 mmol/24h)
Children	13 - 38 mg/24h	(0,14 – 0,42 mmol/24h)

It is suitable that every laboratory determine its reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity: The Oxalate Reagent is linear up to 2,00 mmol/L.
For concentrations ≥ 2 mmol/L, dilute the sample 1:2 with distilled water, repeat the determ. and multiply the result $\times 2$.

Sensitivity: The minimum detectable is 0,02 mmol/L.

Within-run Precision:

	Mean (mg/24h) \pm 2s	CV %
Serum 1	30,4 \pm 3,2	2,8
Serum 2	73,9 \pm 4,3	1,9

Run-to-run (Day-to-day) Precision:

	Mean (mg/24h) \pm 2s	CV %
Serum 1	30,2 \pm 4,2	3,9
Serum 2	73,2 \pm 5,4	2,2

Interferences: See References point 2.

Correlation: A group of 20 urine from 8 to 76 mg/24h was assayed by this procedure and using a similar commercially available **Oxalate** Reagent.
Comparison of the data gave following results:

Linear regression equation $Y = 1,0093x - 0,12$
Correlation coefficient $r = 0,9956$ $n = 20$

NOTE

- 1.A proportional. variation of the reaction volumes does not change the result.
2. We suggest do not mix Reagents from different Production lots.
3. For concent. of Oxalate $\geq 2,00$ mmol/L, dilute the sample 1:2 (1+1) **with distilled water**, repeat the determination and multiply the result $\times 2$.
4. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Oxalate (see References 2.).
5. PAY ATTENTION!
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
6. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
7. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S., Effect of drugs on Clinical Lab. Test, 5th Ed. AACC Press (2000).

Ver. 2007/12