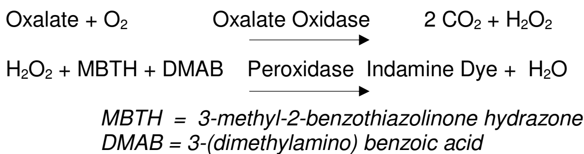
OXALATE

(Quantitative Enzymatic Color Test)

Cat.No	Package Size		
	R1	R2	Sample Diluent
175 080	10 x 10 ml	5 x 2 ml + 2 x 10 ml Standard	5 x 100 ml
175 081	2 x 10 ml	1 x 2 ml + 4 ml Standard	1 x 100 ml
175 082	Sample Purifier Tubes 20 pieces		

METHOD / TESTPRINCIPLE

Enzymatic colorimetric Test :



Oxalate + O_2 $\xrightarrow{\text{Oxalate Oxidase}}$ 2 CO_2 + H_2O_2

H_2O_2 + MBTH + DMAB $\xrightarrow{\text{Peroxidase}}$ Indamine Dye + H_2O

MBTH = 3-methyl-2-benzothiazolinone hydrazone
DMAB = 3-(dimethylamino) benzoic acid

REAGENTS COMPOSITION (concentration in test):

R1 :	DMAB	3.2 mmol/L
	MBTH	0.22 mmol/L
	Buffer	pH 3.1
R2 :	Oxalate Oxidase	3.0 kU/L
	Peroxidase	100 kU/L
Sample Diluent :		
	EDTA	10 mmol/L
	Buffer	pH 7.6
Standard :		
	Oxalate	0.5 mmol/L

Additional Reagent (Cat. No 175 082)
not included in the kit :
Sample Purifier tubes Activated Charcoal

STABILITY OF REAGENTS

When stored at 2-8° C and protected from light and contamination, the reagents are stable up to the expiry date printed on the labels. Do not freeze !

PREPARATION AND STABILITY OF WORKING REAGENTS

R1 : Reconstitute Substrate with 10 ml of dist. water.
R2 : Reconstitute Start Reagent with 2 ml of dist. water .

Stability of R1 : 2 weeks at 2-8°C
R2 : 5 weeks at 2-8°C

Sample Diluent is ready to use

SAMPLES

Urine

REFERENCE VALUES

	mg/24 hrs	mmol/24 hrs
Adult Males	7 - 44	0.08 - 0.49
Adult Females	4 - 31	0.04 - 0.32
Children	13 - 38	0.14 - 0.42

PROCEDURE

Sample preparation:

1. Set up a series of labelled tubes for urine samples and controls.
2. Pipette 5 ml or any suitable volume of urine samples and controls into ***Sample Purifier Tubes.***
3. Add equal volume (as in step 2) of *sample diluent* into each tube and mix for approximately 5 min by intermittent mixing (rotator mixer is recommended).
4. Check the pH, it should be between 5.0 and 7.0 , otherwise adjust the pH using 1N hydrochloric acid and/or 1N sodium hydroxide
5. Centrifuge tubes for 5 min at 2000 rpm (1500 g) or use filtration.
   Determine oxalate concentration in the supernatants or filtrates as described below.

Determination of Oxalate

1. Warm oxalate reagents to assay temperature (preferably 25°C or 30°C or 37 °C).
2. Label tubes for *reagent blank*, *standard*, *controls* and *urine samples*
3. Pipette 1 ml **R1** into each tube.
4. Pipette 50 µl of *supernatants* (or *filtrates)* , to respective tubes. Add 50 µl *deionized water* to reagent blank tube and 50 µl *standard* to tube labelled standard.
5. Pipette 0.1 ml of **R2** into each tube and immediately mix by gentle inversion.
6. Incubate the tubes at desired temperature for 5 minutes.
7. Read absorbances ***(A)*** of *blank, standard, controls* and *urine samples* at 590 nm.
8. Determine the corrected absorbances ***(ΔA)*** of standard, controls and sample by subtracting *reagent blank absorbance* from the *absorbance* readings of *standard, controls* and *urine samples.*

Calculation

$$\textit{Oxalate (mmol/L)} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times 0.5 \times 2$$

0.5 = Concentration (mmol/L) of oxalate in standard
2 = Dilution factor

Quantity of Oxalate excreted during 24 hrs *=*
Oxalate (mmol/L) x Volume of urine (Litres)

QUALITY CONTROL

For quality control use adequate control materials.

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