H-1117 Budapest,
Budafoki street 111-113
Tel.: +36-1-205-3617
Tel./Fax: +36-1-205-3616

TOTAL ANTIOXIDANT CAPACITY (TAOC)

CE

STABLE REAGENT

Kódszám: 48661
Kiszerelés: 4x30 ml (4×1 ml + 1x4 ml + 1x120 ml + 1x2 ml)

Reagent kit to determine the plasma or serum total antioxidant capacity (DPPH method)
The formation of reactive oxygen intermediers in the human body is a natural progress. The damaging effect of the free radicals can be avoid in the human body by the antioxidant mechanisms to some extent however if the balance is shifted into a greater number of free radicals oxidative stress may occur.
Free radicals have significant role in ageing, inflammations, carcinogenesis and liver diseases.
The determination of total antioxidant capacity is suitable for screening tests because the total antioxidant capacity of the plasma can decrease in case of lack of vitamins, inflammations, tumors and damage of immuno-processes and also for monitorising the efficiency of treatments.

Principle
The DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical. The absorbance of DPPH solution decreases in the presence of antioxidant molecules. There are two ways for neutralize this radical, proton or electron transfer. The reaction can be measured with spectrophotometer at 540 nm. The change in the absorbance is proportional with the antioxidant capacity of the sample.

Reference values
Serum: >0,5 mmol/l TEAC
(Trolox Equivalent Antioxidant Capacity)
It is recommended that each laboratory should assign its own normal range.

Reagents
1. Reagent (R1): DPPH freeze dried
2. Reagent (R2): DMSO (solvent for R1)
3. Reagent (R3): buffer for R1+R2 dilution
Tris HCl
detergent
4. Reagent (R4): Standard. See the insert of the standard.

Safety instructions:
Reagent 1:
X, Harmful
R42/43 May cause sensitization by inhalation and skin contact
S22 Do not breathe dust.
S36/37 Wear suitable protective clothing and gloves.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
Reagent 3:
X, Irritant
R36 Irritating to eyes
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

Sample
Serum, plasma, erytrocyte, tissue suspension, wine, beer, fruits, etc.
For urine, wine and fruit sample the recommended dilution ratio is 1:5.

Stability
Avoid from light! Without opening and store at 2-8 °C until the expire date.

PROCEDURE

The method is two-reagent.

First reagent (FR): R3 buffer, ready to use.
Second reagent (SR): Add exactly 1 ml of R2 solvent to one vial R1. Wait until it dissolve (couple of minutes). When ready, add exactly 5 mls of R3 buffer to this R1+R2 solution.

Stability
First reagent (FR): at 2-8°C 4 weeks
Second reagent (SR): avoid from light at 2-8°C 9 days

Assay conditions
Wavelength: 540 (510-550) nm
Temperature: 37°C
Cuvette: 1 cm light path
Method: Endpoint (decreasing)
Reading: against sample blank

Procedure

	sample	standard
FR	1 ml	1 ml
sample	40 µl	
standard		40 µl

Mix and after 1 min incubation read the absorbance (A1).

SR	250 µl	250 µl

Mix and after 5 mins incubation read the absorbance against sample blank (A2). ΔA=A2-A1

Calibration: (DPPH method, at 37 °C)
S1: Distilled water
S2: TAOC standard
Ajánlott új kalibrációt végezni:
- after reagent lot change,
- as required following quality control procedures.

Calculation

$$\frac{\Delta A_{Sample}}{\Delta A_{Calibrator}} x C_{Calibrator} = C_{Sample}$$

A=absorbance
C=concentration

Quality control
A quality control program is recommended for all clinical laboratories. Recommended controls: Diagnosticum Ltd DunaCont N (Cat.:Dcon-N) and DunaCont P (Dcon-P). Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCE DATA

The following data were obtained using Cobas Mira Plus analyzer

Linearity
The test is linear up to 4,1 mmol/l.

Sensitivity
It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used.
The estimated lowest detectabled concentration is 0,07 mmol/l.

Precision

Reproducibility			
	average conc.	SD	CV %
1. sample	0,47	0,04	7,78
2. sample	2,40	0,26	10,72

Repeatability			
	average conc.	SD	CV %
1. sample	0,21	0,017	8,25
2. sample	1,79	0,063	3,54

Correlation
Comperative studies were done to compare our reagent with another commercial bilirubin total test.
The results are detailed below:
Correlation coefficient: r=0,9309
Linear regression: y=1,0115x-0,4632
(x=other commercial reagent, y=own reagent)

Specificity

Hemoglobin up to 2,5 g/l, glucose, bilirubin, lipid, ascorbate don't interefer with the test.

Note
Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solution and reagents described above for any purpose other than descsribed herein.

The R2 component can be freezed under 19°C, however it does not affect the reagent quality. In this case leave this reagent at room temperature until it melts.

For in vitro and research purposes.

A címkéken a következő szimbólumok találhatóak:

IVD For in vitro diagnostic
Use by (last day of the month)
Temperature limitation
LOT Batch Code
REF Code

References:
Blois, M., 1958. Antioxidant determination by the use of a stable free radical. Nature 181, 1199–1200.