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Instructions for use

TAC Fast Track

REF **DM P-4100**

96

+2 +8 °C

RUO For Research use only- Not for use in diagnostic procedures

Total Antioxidative Capacity

Fast colorimetric/photometric test for the quantitative determination of the **t**otal **a**ntioxidative **c**apacity (TAC) in serum and EDTA-plasma.

1. Principle of the test

The determination of the total antioxidative capacity is based on the reaction of peroxides with peroxidase followed by a colour reaction of the chromogenic substrate tetramethylbenzidine. Its blue colour turns to yellow after addition of the stop solution and can be measured photometrically at 450 nm. (Alternatively kinetic measurements at 600 nm are possible, if end point measurement is not wanted.) In case of end point measurement (use of stop solution) samples with absorptions at 450 nm like sera have to be assayed by subtraction of initial absorption. Quantification is achieved by serial dilutions of a standard Antioxidant-solution.

2. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.

It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.

The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.

The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.

All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.

All reagents, however, should be treated as potential biohazards in use and for disposal.

3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiration date shown on the kit labels. Do not mix various lots of any kit component within an individual assay.

4.1 Contents of the kit

DM P-0013	ASSAY-BUFF	**Assay Buffer*[1]*[2]**	1 x 50 mL	ready for use
DM P-0035	PEROXIDE	**Peroxide***	1 x 1 mL	ready for use
DM P-0036	PEROXIDASE	**Peroxidase***	1 x 1 mL	diluted peroxidase, ready for use
DM P-0055	SUBSTRATE	**Substrate***	1 x 1 mL	ready for use, containing a solution of TMB
DM P-0080	STOP-SOLN	**Stop Solution**	1 x 6 mL	ready for use, containing 1 M H_2SO_4
BA D-0032	96	**Microtiter Plate**	1 x 96 wells	12 strips, 8 wells each, break apart
DM P-4101	STANDARD A	**Standard A**	1 x 1 mL	ready for use
DM P-4102	STANDARD B	**Standard B**	1 x 1 mL	ready for use
DM P-4103	STANDARD C	**Standard C**	1 x 1 mL	ready for use
DM P-4104	STANDARD D	**Standard D**	1 x 1 mL	ready for use
DM P-4105	STANDARD E	**Standard E**	1 x 1 mL	ready for use
DM P-4151	CONTROL 1	**Control 1**	1 x 1 mL	ready for use
DM P-4152	CONTROL 2	**Control 2**	1 x 1 mL	ready for use

***[1] Reagent mixture A:**
Mix **10 ml of Assay buffer** and **10 µl** of **Peroxide**.
The reagent mixture A allows 96 determinations and is stable for **15 Minutes!**

***[2] Reagent mixture B:**
Mix **5 ml of Assay buffer** , **50 µl of Substrate** and **5 µl** of **Peroxidase**.
The reagent mixture B allows 96 determinations and is stable for **15 Minutes!**

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1.000µL), - ELISA reader capable of reading absorbance at 450 nm, - Vortex mixer, - Distilled water

5. Sample collection and storage

Serum and EDTA Plasma
Freshly prepared serum or EDTA plasma samples are mandatory. Do not use samples which were stored for longer than 30 minutes at room temperature! Haemolytic and lipemic samples should not be used for the assay. Samples can be stored up to 36 hours at 2 - 8 °C. For a longer period (up to 2 weeks) the samples should be stored at - 20 °C.

⚠ *Only optically clear samples may be used. Fines (kryo proteins) need to be removed by centrifugation (min. 5 min at 10.000xg). Heparin plasma and blood may not be used for this assay.*

6. Test procedure

Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Duplicates are recommended.

6.1 Preparation of reagents

Reagent mixture A
Mix 10 ml of Assay Buffer and 10 µl of Peroxide (sufficient for 96 determinations).

Reagent mixture B
Mix 5 ml of Assay Buffer, 50 µl of Substrate and 5 µl of Peroxidase (sufficient for 96 determinations).

⚠ *(If less than 96 determinations are needed, prepare smaller amounts of Reagent mixture A + B*

⚠ *The reagent mixture* A + B *has to be prepared freshly prior to the assay (not longer than 15 minutes in advance). Discard after use!*

6.3 TAC Assay

1.	Pipette **25 µL** of the **standards, controls and samples** into the respective wells of the **Microtiter Plate**.
2. ⚠	Pipet **100 µL** of **reagent mixture A** (refer to 6.1*)* into all wells. *Pipetting should be completed within 1 minute!*
2. ⚠	Pipet **50 µL** of **reagent mixture B** (refer to 6.1*)* into all wells. *Pipetting should be completed within 1 minute!*
3.	Incubate **exactly** for **20 minutes** at **2 – 8 °C**.
4. ⚠	Pipette **50 µL** of the **Stop Solution** to each well of the microtiter plate. *Pipetting should be completed within 1 minute!*
5.	**Read** the absorbance of the solution in the wells, using a microplate reader set to **450 nm**.

7. Calculation of results

Concentration of the standards	A	B	C	D	E
TAC (mmol/L)	0	0.375	0.75	1.5	3

⚠ *The concentrations of standards are given in equivalents of* Trolox ($C_{14}H_{18}O_4$) *(mmol/L).*

The calibration curve is obtained by plotting the extinction values measured for the standards (linear, y-axis) against the corresponding concentrations (linear, x-axis). The results for unknowns can be calculated using a linear fit.

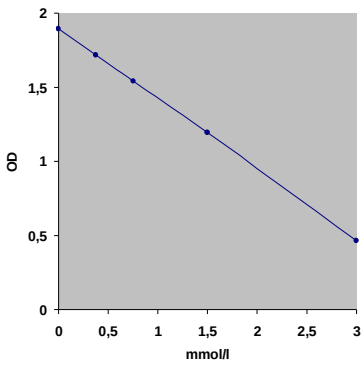
7.1 Quality control

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC Report.

8. Assay characteristics

Expected values	**TAC**	< 1 mmol/L ⇔ antioxidative capacity too low 1 – 1.3 mmol/L ⇔ borderline antioxidative capacity > 1.3 mmol/L ⇔ sufficient antioxidative capacity	
Analytical Sensitivity (Limit of Detection)		Mean signal (Zero-Standard) - 2SD	
		TAC	0,08 mmol/L
Analytical Specificity		The Test is measurung specific TAC!	

Typical calibration curve



⚠ *Example. Do not use for calculation!*

Precision							
Intra-Assay				**Inter-Assay**			
	Sample	Mean ± SD (mmol/L)	CV (%)		Sample	Mean ± SD (mmol/Ll)	CV (%)
TAC	1	0,8 ± 0,02	2,5	TAC	1	0,8 ± 0,03	3,33
	2	1,6 ± 0,08	5		2	1,59 ±0,11	6,92

		Range	Serial dilution up to	Range (%)
Linearity	TAC	0.4 – 6 mmol/L	1:10	90 – 128

		Mean (%)	Range (%)	% Recovery after spiking
Recovery	TAC	102	87 – 120	

⚠ ***For updated literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE labelled
	Caution		Catalogue number		For research use only!