Manual

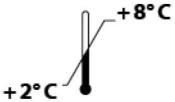
Thiol status

(Sulfhydryl status assay)

Photometric assay for the determination of sulfhydryl status in serum, plasma, urine and synovia

Valid from 20.09.2006

REF K 1800



IVD CE

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Content

1. INTENDED USE

This photometric assay is suitable for the determination of the thiol (sulfhydryl) status (GSH, protein bound and free SH groups) in plasma, serum, urine and synovia. For in vitro diagnostic use only.

2. SUMMARY AND EXPLANATION OF THE TEST

Oxidative stress, or the production of oxygen-centered free radicals, has been hypothesized as the major source of DNA damage that in turn can lead to altered genetic expression, disease, and aging of humans.
Serum protein thiol levels in blood are a direct measure of the in vivo reduction/oxidation (redox) status in humans, because thiols react readily with oxygen-containing free radicals to form disulfides. Moreover, serum thiols also reflect DNA repair capacity and the possible eventual accumulation of genetic damage, since a key DNA repair enzyme, poly ADP-ribose polymerase (PARP), is thiol/disulfide redox regulated.
Serum protein thiols can possibly be used to estimate individual aging status. Data from Banne et al. (2003) strongly confirm an important role of oxidative stress in human disease development, and identify serum thiol status as a potential biochemical endpoint useful in the assessment of aging.

3. PRINCIPLE OF THE TEST

When the sample is added to the reaction buffer A together with the reaction buffer B, free and bound SH groups from the sample undergo a reaction, that results in a yellow colored product with an absorption maximum at 412 nm. The quantitation is performed by the delivered calibrator.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
K 1800KA	CAL	Calibrator (lyoph. 1 ml; 1000 µmol/L)	4 vials
K 1800RA	REABUF A	Reaction buffer A	1 x 24 ml
K 1800RB	REABUF B	Reaction buffer B	1 x 2,4 ml
K 1800MTP	PLATE	Microtiter plate	1 plate

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water
- Precision pipettes calibrated to deliver 10-1000 µl
- ELISA-reader
- Incubation chamber for 37 °C

6. PREPARATION AND STORAGE OF REAGENTS

All reagents are stable at 2-8 °C up to the expiry date stated on of the label.

Preparation of the reagents

The REABUF A (reaction buffer A) has to be used undiluted
The REABUF B (reaction buffer B) has to be used undiluted

Reconstitution of the calibrator
The CAL (calibrator) must be reconstituted in 1000 µl bidistilled water. The reconstituted calibrator is stable at 2-8°C or at –20°C for one week.

7. PRECAUTIONS

- For in vitro diagnostic use only.
- Reagents should not be used beyond the expiration date shown on kit label.
- Do not mix reagents of different lots.
- The assay should always be performed according the enclosed manual.
- Seal the cavities with plastic foil during incubation.

8. SPECIMEN COLLECTION AND PREPARATION

Plasma, serum, urine, sonovia

- Whole blood is not suited for this test.
- Lipaemia and haemolysis interfere with the test system. Such samples should not be measured.
- Samples with visible amounts of precipitates should be centrifuged (5 min at 10000 g) prior to measurement and the resulting supernatant is used in the test.

9. ASSAY PROCEDURE

Procedural notes

- The quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. Immundiagnostik can therefore not be held reliable for any damage resulting from this.
- The assay should always be performed according the enclosed manual.

Testpreparations

- The microtiter plate is ready to use.
- To ensure the reproducibility of the measurement, the given incubation time and temperature should be followed strictly.

Test initiation

1. Pipet 20 µl of sample, calibrator (CAL) and blank value (bidistilled water) in duplicates in appropriate wells.
2. Add 200 µl of REABUF A (reaction buffer A).
3. Measurement 1: Read the absorption of the samples in the ELISA reader at 405 nm.
4. Add 20 µl of REABUF B (reaction buffer B).
5. Incubate for 30 min at 37° C (seal the cavities with plastic foil).
6. Measurement 2: is performed immediately after the incubation at 405 nm in the ELISA reader.

10. EVALUATION OF RESULTS

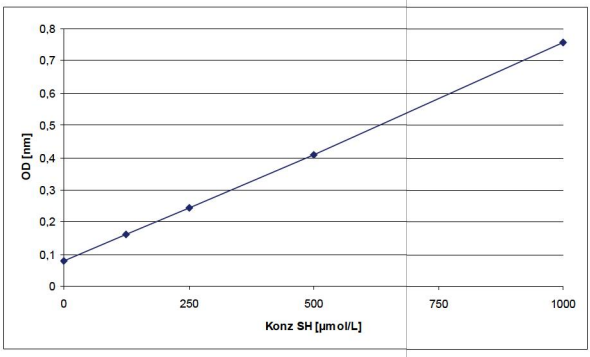
Calculation

The difference between measurement 1 and 2 is directly proportional to the thiol- (sulfhydryl) status of the sample. For evaluation of the measured samples and the calibrator, the optical densities of measurement 1 are subtracted from the optical densities of measurement 2.
Samples and controls are then calibrated by the use of the calibrator (concentration is given on the label).

$$\text{Concentration of the sample [µmol/l]} = \frac{\Delta \text{ OD x Concentration Calibrator [µmol/l]}}{\Delta \text{ OD Calibrator}}$$

Note: This linear standard curve is only for demonstration purposes. A standard curve should be generated for each set of samples assayed. Because of the linearity, only one-point calibration using the purchased calibrator is sufficient.

Standard curve (for demonstration purposes)



11. TESTPERFORMANCE

Reference values

Serum, plasma: 430 – 660 µmol / L (2SD)

We recommend each laboratory to establish its own normal range. The values mentioned above are only for orientation and can deviate from other published data.

Controls

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

12. LITERATURE

Banne AF et al. (2003) Anti Aging Med 6: 327-34
Belch JJ et al. (1991) Br Heart J May; 65(5):245-248
Dabrowski A, Gabryelewicz A (1992) Int J Pancreatol Dec; 12(3): 193-199
Collier A et al. (1979) Diabet Med 7(1): 27-30
Ellmann G, Lysko H (1979) Anal Biochem; Feb; 93(1): 98-102
Hu-ML: Methods-Enzymol. 1994; 233: 380-385
Jocelyn PC (1987) Methods Enzymol ; 143: 44-67
Langley SC et al. (1993) Pediatr Res; Mar; 33(3): 247-250
Miesel R, Zuber M (1993) Inflammation. Oct; 17(5): 551-561
Nguyen TT et al. (1993) J Burn Care Rehabil; Nov-Dec; 14(6): 602-609
Riddles PW et al. (1983) Methods Enzymol 91: 49-60
Sitton NG (1986) Wright-V: Rheumatol Int 6(6): 251-254

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- The test components contain organic solvents. Contact with skin or mucous membranes has to be avoided.
- All reagents in the test package are to be used for research only.
- The reagents should not be used after the date of expiry stated on the label.
- Do not interchange different lot numbers of any kit component within the same assay.
- The guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.

Used Symbols:

 Temperature limitation

REF Catalogue Number

IVD In Vitro Diagnostic Medical Device

 Contains sufficient for <n> tests

 Manufacturer

 Use by

LOT Lot number

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