Manual

Thiol status

Sulfhydryl status assay

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

Valid from 2022-05-02

REF **KR1800**

Σ 96

+8 °C
+2 °C

RUO

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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 research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Oxidative stress, or the production of oxygen-centered free radicals, has been hypothesised as the major source of DNA damage that in turn can lead to altered genetic expression, disease, and ageing 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 ageing status.

Possible research areas

- Pancreatitis
- Rheumatoid and reactive arthritis
- Systemic *Lupus erythematodes*
- Scleroderma, mixed connective tissue disease
- Carcinomas
- AIDS
- Heart failure
- Lung diseases
- Diabetic neuropathy

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR1800	PLATE	Microtiter plate	12 x 8 wells
KR1800	CAL	Calibrator, lyophilised (1 000 µmol/l)	4 vials
KR1800	CTRL 1	Control, lyophilised	4 vials
KR1800	CTRL 2	Control, lyophilised	4 vials
KR1800	REABUF A	Reaction buffer A	1 x 24 ml

Cat. No.	Label	Kit components	Quantity
KR1800	REABUF B	Reaction buffer B	1 x 2,4 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1 000 µl single-use tips
- Incubation chamber for 37 °C
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the calibrator:** The **lyophilised calibrator (CAL)** is stable at **2–8 °C** until the expiry date stated on the label. Before use, the CAL has to be reconstituted with **250 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. The **calibrator** (reconstituted CAL) **can be stored at -20 °C for 7 days.**
- **The lyophilised controls 1 and 2 (CTRL 1 and 2)** are stable at **2–8 °C** until the expiry date stated on the label. Before use, the CTRLs have to be reconstituted with each **250 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. The **controls 1 and 2** (reconstituted CTRL 1 and 2) **can be stored at -20 °C for 7 days.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

- 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 10 000 *g*) prior to measurement and the resulting supernatant is used in the test.

7. ASSAY PROCEDURE

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.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of calibrator/sample/controls on a protocol sheet.

Take as many microtiter strips as needed from kit. Store unused strips covered at 2–8 °C. Strips are stable until expiry date stated on the label.

We recommend to carry out the tests in duplicate.

1.	Pipet **20 µl** of **sample**, **calibrator** and **controls** in the corresponding wells.
2.	Add **200 µl reaction buffer A** (READUF A).
3.	**Measurement 1**: read the absorption of the samples in the microtiter plate reader at **405 nm**.
4.	Add **20 µl reaction buffer B** (READUF 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 microtiter plate reader.

8. RESULTS

The difference between measurement 1 and 2 is directly proportional to the thiol-(sulfhydryl) status of the sample. For evaluation, the optical densities of measurement 1 are subtracted from the optical densities of measurement 2.

Samples and controls are then calculated by the use of the calibrator:

$$\text{Sample concentration [µmol/l]} = \frac{\Delta OD \times \text{calibrator concentration [µmol/l]}}{\Delta OD \text{ calibrator}}$$

9. LIMITATIONS

Whole blood is not suited for this test.

10. QUALITY CONTROL

Immundiagnostik recommends the use of external controls for internal quality control, if possible.

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

Reference range

We recommend each laboratory to establish its own reference range.

11. PRECAUTIONS

- All reagents in the kit package are for research use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.

12. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories should be followed.
- 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 incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

14. REFERENCES

1. Banne, A.F., Amiri, A. & Pero, R.W., 2003. Reduced level of serum thiols in patients with a diagnosis of active disease. *Journal of anti-aging medicine*, **6**(4), pp.327–34.
2. Belch, J.J. et al., 1991. Oxygen free radicals and congestive heart failure. *British heart journal*, **65**(5), pp.245–8.
3. Himmelfarb, J. et al., 2004. Oxidative stress is increased in critically ill patients with acute renal failure. *Journal of the American Society of Nephrology : JASN*, **15**(9), pp.2449–56.

Used symbols:


Temperature limitation

Catalogue number

For research use only

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet