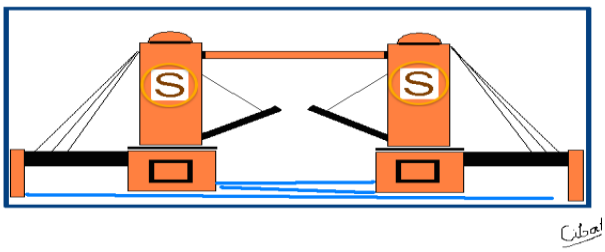
Thiol Disulfide Homeostasis

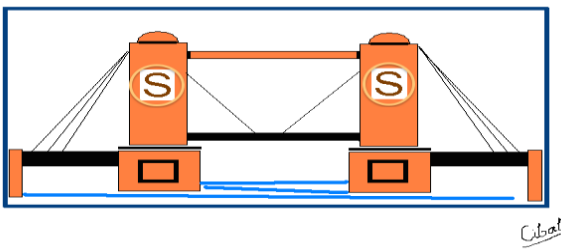
Thiols, also known as mercaptans, are a class of organic compounds that contain a sulfhydryl group (–SH) composed of a sulphur atom and a hydrogen atom attached to a carbon atom. The plasma thiol pool is mainly formed by albumin thiols, protein thiols and slightly formed by low-molecular-weight thiols such as cysteine (Cys), cysteinylglycine, glutathione, homocysteine and γ- glutamylcysteine.

Thiols (RSH) can undergo oxidation reaction via oxidants and form disulphide (RSSR) bonds. A disulphide bond is a covalent bond; the linkage is also called a SS-bond or disulphide bridge. Under conditions of oxidative stress, the oxidation of Cys residues can lead to the reversible formation of mixed disulphides between protein thiol groups and low-molecular-mass thiols. The formed disulphide bonds can again be reduced to thiol groups; thus, dynamic thiol–disulphide homeostasis is maintained.

Dynamic thiol disulphide homeostasis status has critical roles in antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activity and transcription factors and cellular signalling mechanisms. Moreover, dynamic thiol disulphide homeostasis is being increasingly implicated in many disorders. There is also a growing body of evidence demonstrating that an abnormal thiol disulphide homeostasis state is involved in the pathogenesis of a variety of diseases, including diabetes [1-3], cardiovascular disease [4-10], cerebrovascular disase [11], malignacies [12], ankylosing spondylitis [13], preeclampsia [14,15], Alzheimer's disease [16], migraine [17], infections [18,19], and various disorders. Therefore, determination of dynamic thiol disulphide homeostasis can provide valuable information on various normal or abnormal biochemical processes. This easy, practical, fully automated and also optionally manual spectrophotometric assay can be used to determine plasma dynamic thiol/disulphide homeostasis.

Thiol Disulfide Homeostasis Tests





- **Native Thiol Status (-SH)**
- **Dynamic Disulfide Status (-S-S-)**
- **Total (Oxidized and Reduced) Thiol Status (-SH + -S-S-)**
- **Reduced Thiol Ratio [(-SH)/ (-SH + -S-S-)]X 100**
- **Oxidized Thiol (disulfide) Ratio [(-S-S-)/ (-SH + -S-S-)] X 100**
- **Thiol Oxidation Reduction Ratio [[(-SH)/ (-S-S-)]X 100**

Priniciple of the Assay

Reducible disulphide bonds were reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde, and all thiol groups including reduced and native thiol groups were determined after the reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB). Half of the difference between the total thiols and the native thiols was recorded as the dynamic disulphide amount. After the native thiols (SH) and total thiols were determined, disulphide (SS) amounts, disulphide/total thiol percent ratios (SS/SH+SS), disulphide/native thiol percent ratios (SS/SH), and native thiol/total thiol percent ratios (SH/SH+SS) were calculated [20].

Application parameters of the assays

Sample volume: 10 µL

R1 volume (for total –SH): 10 µL

R1' volume (for native –SH): 10 µL

R2 (2') volume: 110 µL,

R3 (3') volume: 10 µL.

Wavelength (main wavelength): 415 nm, secondary wavelength 700 nm, (optionally bichromatic).

Reading point: End-point, increasing measurement; the first absorbance is taken before the mixing of R2 and R3 and the last absorbance is taken when the reaction trace draws a plateau (assay duration is about 10 min).

Calibration type: Linear

The disulphide parameter is a value which can be calculated automatically as half of the difference of the two measured values. The assays can also be performed by manually using spectrophotometers or multiwell readers. All volumes of the samples and reagents must be increased at the same ratio. Use of a second (side) wavelength is optional.

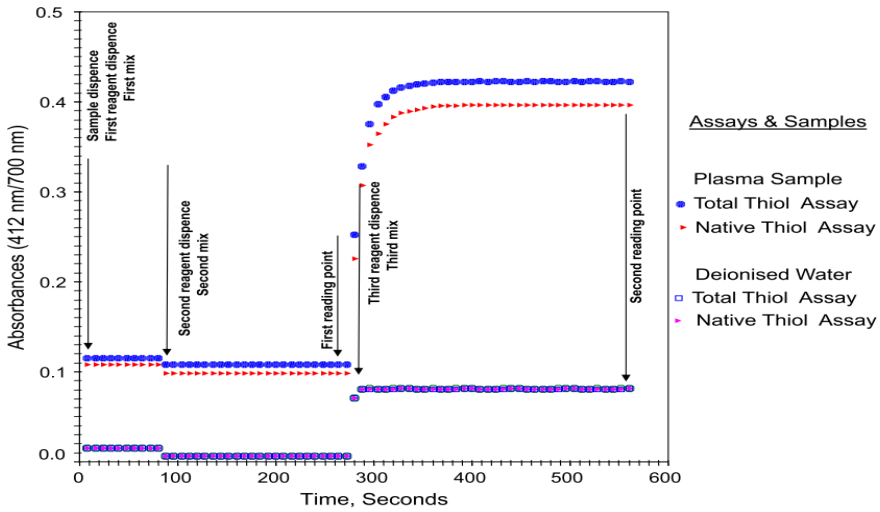


Figure. Reaction kinetics of the assays.

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