QuantiChrom™ Sulfate Assay Kit (DSFT-200)

Quantitative Turbidimetric Sulfate Determination at 600 nm

DESCRIPTION

INORGANIC SULFATE is one of the most abundant anions in mammalian plasma. Sulfate plays important physiological roles in activating and detoxifying xenobiotics, steroids, neurotransmitters, and bile acids. Sulfate is needed for the biosynthesis of glycosaminoglycans, cerebroside sulfate, and heparin sulfate. Undersulfation of cartilage proteoglycans has been associated with human inherited osteochondrodysplasia disorders. In mammals, sulfate homeostasis is regulated by the kidney. The majority of filtered sulfate is absorbed in the proximal tubules, and only 5–20% of the filtered load is excreted into the urine.

Simple, direct and automation-ready procedures for quantitative determination of inorganic sulfate find wide applications in research and drug discovery. BioAssay Systems' sulfate assay kit is designed to measure sulfate concentration in biological fluids such as serum and urine. The improved method utilizes the quantitative formation of insoluble barium sulfate in polyethylene glycol. The turbidity measured between 540 and 610nm is proportional to sulfate level in the sample.

KEY FEATURES

Sensitive and accurate. Detection range 0.02 mM (0.19 mg/dL) to 2 mM (19.2 mg/dL) sulfate in 96-well plate assay.
Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 5 min.

APPLICATIONS:

Direct Assays: inorganic sulfate in serum and urine.
Pharmacology: effects of drugs on sulfate metabolism.

KIT CONTENTS (200 tests in 96-well plates)

Reagent A: 25 mL **Reagent B: 2.4 g Powder**
TCA Reagent: 25 mL **Sulfate Standard: 1 mL 60 mM**

Storage Conditions. The kit is shipped and stored at room temperature. Shelf life of 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Treatment: Urine samples should be diluted 10-fold in deionized water prior to assay.

Fresh serum or plasma (non-hemolyzed) samples can be either assayed immediately, or frozen for future tests. Samples should be deproteinated as follows: mix 200 µL sample and 100 µL TCA Reagent in a 1.5-mL Eppendorf tube. Spin down protein precipitates 5 min at 14,000 rpm on a table centrifuge. Transfer 200 µL supernatant for assay.

Procedure using 96-well plate:

1. **Standards**. Prepare a 2.0 mM Premix by mixing 20 µL 60 mM Sulfate Standard with 580 µL dH2O. Dilute Premix as follows.

No	Premix + H_2O	Sulfate (mM)
1	200 µL + 0 µL	2.0
2	100 µL + 100 µL	1.0
3	50 µL + 150 µL	0.5
4	0 µL + 200 µL	0

Transfer 200 µL Standards into separate wells of a clear flat-bottom 96-well plate. *Note: when deproteination is required (i.e. serum or plasma), treat the standards by adding 100 µL TCA Reagent to 200 µL of each standard, mix and transfer 200 µL of the resulting standard into separate wells.*

Transfer 200 µL Samples (see above for Sample Treatment) into separate wells of the plate.

2. **Working Reagent**. The Working Reagent (WR) must be prepared fresh and used within 1 hour after reconstitution.

Prepare enough WR for all samples and standards (100 µL WR per assay well) by mixing 95 mg Reagent B per mL Reagent A, i.e. 10 assay wells, mix 95 mg Reagent B with 1 mL Reagent A. Vortex for at least 1 min to ensure complete dissolution of the powder and incubate the reconstituted Working Reagent for 10 min before use.

Use a multi-channel pipettor, add 100 µL Working Reagent to each assay well. Tap plate to mix well. If TCA precipitation was required, mixing the samples and standards with the WR can be improved by pipetting up and down once.

3. Incubate 5 min at room temperature and read optical density at 540-610nm (600nm).

CALCULATION

Sulfate concentration (mM) in the sample can be calculated as follows,

$$[\text{Sulfate}] = \frac{OD_{SAMPLE} - OD_{H2O}}{\text{Slope}} \times n \quad (\text{mM})$$

OD_{SAMPLE} and OD_{H2O} are the OD values of the sample well and the water well (Standard #4), respectively. n = 10 for urine.
Conversions: 1 mM sulfate equals 9.61 mg/dL or 96.1 ppm.

GENERAL CONSIDERATIONS

This procedure can be scaled up to assays in a cuvette.

The following compounds have been tested and do not interfere: 400 mM sodium chloride, 500 mM urea, 5 mM sodium phosphate, 4 mM sodium citrate, 1.5 mM sodium EDTA.

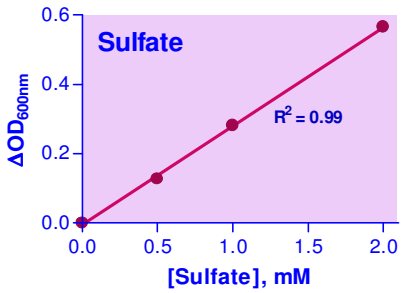
Ester sulfate can be determined following a digestion step (Lundquis, P. et al. (1980). Clin. Chem. 26:1178-81). This will allow quantification of total inorganic and ester sulfate in a sample.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories, clear flat-bottom 96-well plates and plate reader, or cuvets and spectrophotometer.

EXAMPLES:

Duplicate assays for rat serum, fetal bovine serum, human serum, and human urine gave sulfate content of 1.54 ± 0.07, 1.69 ± 0.09, 0.48 ± 0.01 and 10.5 ± 0.09 mM (n = 2).



Standard Curve in 96-well plate assay

PUBLICATIONS

1. Majdoub, H et al (2009). Anticoagulant activity of a sulfated polysaccharide from the green alga Arthrospira platensis. Biochim Biophys Acta 1790(10):1377-81.

2. Wang, ZM et al (2011). Structural characterisation and immunomodulatory property of an acidic polysaccharide from mycelial culture of Cordyceps sinensis fungus Cs-HK1. Food Chemistry 125(2): 637-43.

3. Ben Mansour, M et al (2009). Characterization of a novel dermatan sulfate with high antithrombin activity from ray skin (Raja radula). Thromb Res 123(6): 887-94.