

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 plasma¹. 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 urine².

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 glycol³. The turbidity which is measured as optical density between 540 and 610nm (recommended 600nm), is an accurate measure of the sulfate level in the sample.

KEY FEATURES

Sensitive and accurate. Detection range 0.01 mM (0.096 mg/dL) to 1.2 mM (11.5 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. Can be readily automated as a high-throughput assay in 96-well plates for thousands of samples per day.

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: 25 mL**
Sulfate Standard: 2 mL 60 mM sodium sulfate.

Kits are shipped at room temperature.

Storage Conditions: store all reagents at 4°C.

Shelf life: 24 months.

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 20-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 Reagent B 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.

Reagent Preparation: prepare at least 100 µL/well Working Reagent by mixing 2000 µL Reagent A with 2 µL 60 mM sulfate standard. This solution should be vortex (if tube is used) or shaken (if bottle is used) immediately to mix the reagents completely. Prepare 1 mM working Sulfate Standard by mixing 20 µL 60 mM standard with 1180 µL deionized water.

Procedure using 96-well plate:

1. Transfer 200 µL deionized water (blank) and 200 µL 1 mM Sulfate Standard into wells of a clear bottom 96-well plate. Transfer 200 µL sample (serum/plasma supernatant, or urine) in duplicate.
2. Add 100 µL Working Reagent to each well. Tap plate to mix.
3. Incubate 5 min at room temperature and read optical density at 540-

610nm (600nm). Signal is stable for > 60 min.

Procedure using Cuvet:

1. Transfer 600 µL deionized water, 600 µL 1 mM Sulfate Standard, 600 µL sample (serum/plasma supernatant, or urine) into 1.5-mL centrifuge tubes.
2. Add 300 µL Working Reagent. Mix quickly by inversion or vortexing. Transfer contents to cuvetts.
3. Incubate 5 min and read OD_{600nm}.

CALCULATION

The assay is linear up to 1.2 mM sulfate concentration. Sulfate concentration (mM) in the sample can be calculated from the single 1 mM standard as follows,

$$[\text{Sulfate}] = 1 \times (\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}) / (\text{OD}_{\text{STANDARD}} - \text{OD}_{\text{BLANK}}) \times n$$

"1" is the standard sulfate concentration (1mM). *n* is the dilution factor. *n* = 20 for urine samples and 1.5 for serum/plasma samples.

Conversions: 1 mM sulfate equals 9.61 mg/dL or 96.1 ppm.

GENERAL CONSIDERATIONS

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

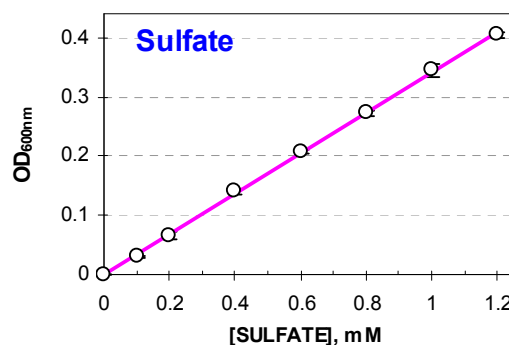
Ester sulfate can be determined using the same method following a digestion step³. This will allow quantification of total sulfate (inorganic and ester sulfate) in biological samples.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories, clear flat-bottom 96-well plates and plate reader, or cuvetts 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).



Calibration curve in 96-well plate

LITERATURE

1. Bolt, M.J.G. et al. (2004). Critical role of vitamin D in sulfate homeostasis: regulation of the sodium-sulfate cotransporter by 1,25-dihydroxyvitamin D3. *Am J Physiol Endocrinol Metab* 287: E744–E749.
2. Becker, E.L. et al. (1960) Renal mechanisms for the excretion of inorganic sulfate in man. *J Clin Invest* 39: 1909–1913.
3. Lundquis, P. et al. (1980). Turbidimetry of inorganic Sulfate, Ester Sulfate, and Total Sulfur in Urine. *CLIN. CHEM.* 26/8, 1178-1181.