ABS_Bio™ Sulfate Detection Kit

(Cat# K149-200; 200 assays; store kit at 4℃)

Introduction

Sulfate is produced in the body from the trans-sulfuration of methionine to cysteine, followed by the oxidation of cysteine to pyruvate and inorganic sulfate. The major end-product of sulfate metabolism in the human body is inorganic sulfate, which is excreted in the urine in free form together with small amounts of its esters with aromatic compounds. The serum sulfate was markedly elevated in patients with chronic nephritis or endotoxemia.

The ABS_Bio™ Sulfate Detection Kit provides a fast, reproducible, colorimetric method for measuring sulfate in urine, serum, plasma and others liquid samples. The improved turbidimetric measurements where sulfate ion is converted to a barium sulfate suspension under controlled stabilizing reagent conditions. The resulting turbidity is determined by a spectrophotometer at OD 600 nm (500-700 nm) and compared with a curve prepared from standard sulfate solution. The kit is supplied with sufficient reagents for 200 tests in 96-well plate assay, linear detection range of 0.01-3 mM. It could easily be modified for use in cuvette-based assays.

Kit Components (200 tests)

Assay Diluent : 35 mL Reagent: 2.2 g Sulfate Standard (60 mM): 2.0 mL 10% TCA: 35 mL

Storage and Handling: Store kit at 4℃. Shelf Life: 12 months after receipt. Warm Reagents to room temperature before use.

Protocol

1. Sample preparation

Serum, plasma and urine samples need simply deproteinization with trichloroacetic acid (TCA) in order to avoid interference. Transfer 200 µL samples into 1.5 mL micro centrifuge tubes, add same volume 10% TCA into the tubes, vortex and quick spin down.

Transfer 150 µL supernatant into a clear flat-bottom 96 well plate in duplicate.

2. Standard Preparation

Vortex and quick spin sulfate standard tube. In a 1.5 mL micro centrifuge tube, add 100 µL 60 mM sulfate standard into 900 µL dH_2O to prepare 6 mM sulfate standard, then following the table to generate 3, 1.8, 0.9, 0.3 and 0 mM sulfate standards.

Sulfate std(µL)	dH_2O(µL)	10% TCA(µL)	final Sulfate (mM)
200	0	200	3
120	80	200	1.8
60	140	200	0.9
20	180	200	0.3
0	200	200	0

Vortex and quick spin down the tubes. Transfer 150 µL supernatant of appropriate standards into duplicate wells in the 96-well plate. The blank control containing dH_2O only.

3. Reaction

Prepare enough working reagents by mixing 10 mg reagent with 150 µL assay diluent for each reaction. Vortex to dissolve the reagent completely.

Then transfer 150 µL prepared working reagent into each well containing the standard and samples, and tap plate lightly to mix well.

4. Measurement

Read absorbance at 600 nm (500-700 nm) for 5 minutes.

5. Calculation

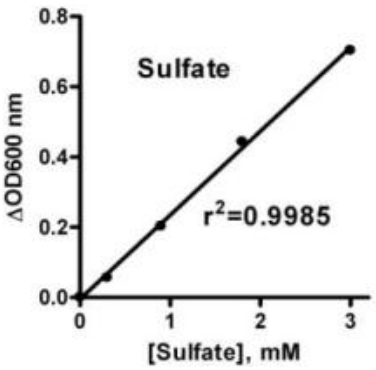
Average the duplicate OD600 nm reading for standard and sample. Subtract the average OD of the blank from the average OD of the standards and plot the result (ΔOD) versus the sulfate concentration of the standards. Determine the slope and calculate the sulfate concentration of samples.

[Sulfate]= (OD_{sample}-OD_{blank})/Slope · *n* (mM) *n* is the sample dilution factor.

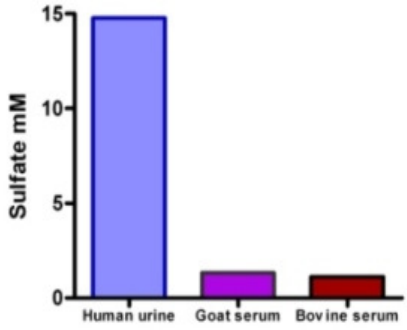
OD_{sample} and OD_{blank} are related optical density of the sample and dH_2O.

Conversions: 1 mM sulfate equals 9.61 mg/dL

Typical Standard Curve



Sulfate standard in 96 wells-plate assay.



Samples in 96 wells-plate assay.

Always run your own standard curves for calculation of results.

Sensitivity and Limit of Detection

The Limit of Detection was determined as 0.01 mM, and linear detection range up to 3 mM in 96-well plate assay. Sensitivity was determined as 0.01 mM. Samples with values above 3 mM should be dilute with dH_2O, re-assayed, and multiply results by dilution factor.

Interferences

D-galactose, D-galactose 6-phosphate, D-glucose were tested in the assay for interference in urine samples. No significant change in the measured sulfate level was observed in spiked samples. Plasma should chose citrate treatment.

The ester sulfate is not hydrolyzed under this condition, to measure ester sulfate please following Lundquist's acid hydrosis method.

References

Lundquist, P. et al. 1980, *Clin Chem* 26:1178-1181
Kolmert A, et al. 2000, *Journal of Microbiological Methods* 41: 179–184
Berglund, F., and Sorbo B., 1960, *Scand J Clin Lab Invest Chem* 12:147-153

Related Products:

ALP Colorimetric Detection Kit (#K109-200)
ALP Fluorimetric Detection Kit (#K110-200)
ALP Substrate Solution (#C8040)
BCIP/NBT Substrate system (#C8041)
TBARS(MDA) Detection Kit (#K145-100)
Glucose Detection Kit (#K188-100)
Glutathione Detection Kit (#K140-100)
Glutathione Peroxidase Detection Kit (#K143-100)
Glutathione Reductase Detection Kit (#K146-100)
HRP Fluorimetric Detection Kit (#K210-100)
Superoxide Dismutase (SOD) Detection Kit (#K129-100
Lactate Dehydrogenase Activity Detection Kit(#K107-100)
Creatinine Detection Kit (#K148-200)
Xanthine Detection Kit (#K131-100)
SOD Activity Detection Kit (#K129-100)
Hydrogen Peroxide Detection Kit (#K125-100)
Lactate Detection Kit (#K207-100)
NAD/NADH Detection Kit (#K120-100)
NADP/NADPH Detection Kit (#K123-100)
Nitric Oxide Detection Kit (#K116-100)
Phosphatase Detection Kit (#K196-500)
Urea Detection Kit (#K158-200)
Alcohol/Ethanol Detection Kit (#K105-100)
Hemoglobin Detection Kit (#K168-200)
Lactate Fluorimetric Detection Kit (#K208-100)
Pyruvate Detection Kit (#K150-100)
Alcohol Dehydrogenase Activity Detection Kit (#K113-100)
Ammonia Detection Kit (#K203-100)
Xanthine Oxidase Activity Detection Kit (#K132-100)