Sulfate Microplate Assay Kit

User Manual

Catalog # CAK1153

(Version 1.2B)

Detection and Quantification of Sulfate Content in Urine, Serum, Plasma, Other biological fluids, Tissue extracts, Cell lysate, Cell culture media Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Inorgainc 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.

Sulfate Microplate 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 glycerol. The turbidity measured at 450nm is proportional to sulfate level in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Substrate	Powder x 1	4 °C
Reaction Buffer	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Substrate: add 5 ml distilled water to dissolve before use. Store at 4 °C. Use within one month.

Standard: add 1 ml distilled water to dissolve before use. The concentration will be 200 mmol/L. Store at 4 °C. Use within one month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into a microcentrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, transfer the supernatant to a new microcentrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, transfer the supernatant to a new centrifuge tube for detection.

3. For serum, plasma or urine samples

Mix 500 µl sample and 500 µl Assay buffer in a microcentrifuge tube. Spin down protein precipitates 5 min at 8000 rpm on a table centrifuge. Transfer the supernatant to a new microcentrifuge tube for detection.

4. For water samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Sample	50 µl	--	--
Standard	--	50 µl	--
Distilled water	--	--	50 µl
Reaction Buffer	100 µl	100 µl	100 µl
Mix.			
Substrate	50 µl	50 µl	50 µl
Mix, record absorbance measured at 450 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{Sulfate (mmol/L)} = C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times n$$

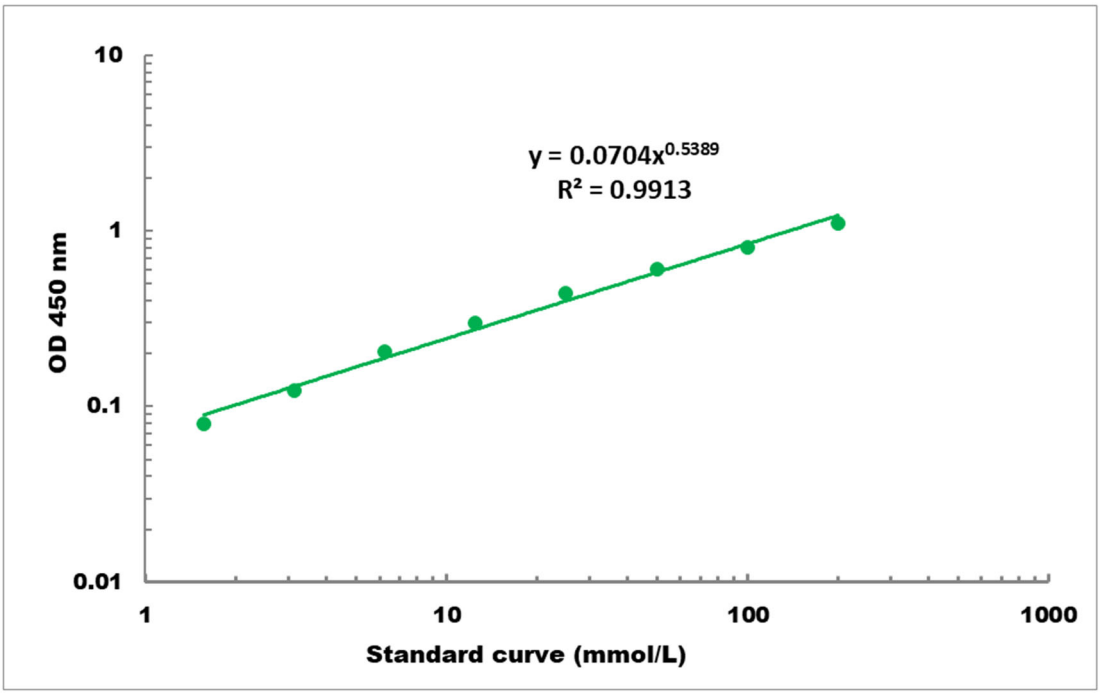
$$= 200 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times n$$

$C_{Standard}$: the concentration of Standard, 200 mmol/L.

n: dilution ratio.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 2 mmol/L - 200 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES