Sulfate

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1153

(Version 1.3B)

Detection and Quantification of SulfateContentin Urine, Serum, Plasma, Other biological fluids, Tissue extracts, Cell lysate, Cell culture mediaSamples.

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

I. INTRODUCTION

InorgaincSulfate 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 bythe 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 Colorimetric 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 inglycerol. The turbidity measured at450nm is proportional to sulfate level in the sample.

II.KIT COMPONENTS

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

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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 200 mmol/L of standardtop solution, store at 4 °C for 1 monthafter reconstitution.Then perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be200/100/50/25/12.5/6.25/3.12mmol/L.

Substrate: Add 5 ml distilled waterto dissolve before use.Store at 4 °C and use within one month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into a microcentrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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 mlAssay 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 urinesamples

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

4.For watersamples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents in the microplate:

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

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times C_{Protein}\times V_{Sample}}(\mu mol/mg)$$

2.2According to the quantity of cells or bacteria

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times N\times(V_{Sample}/V_{Assay})}(\mu mol/10^{4})$$

2.3 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.4According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}\times n(mmol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

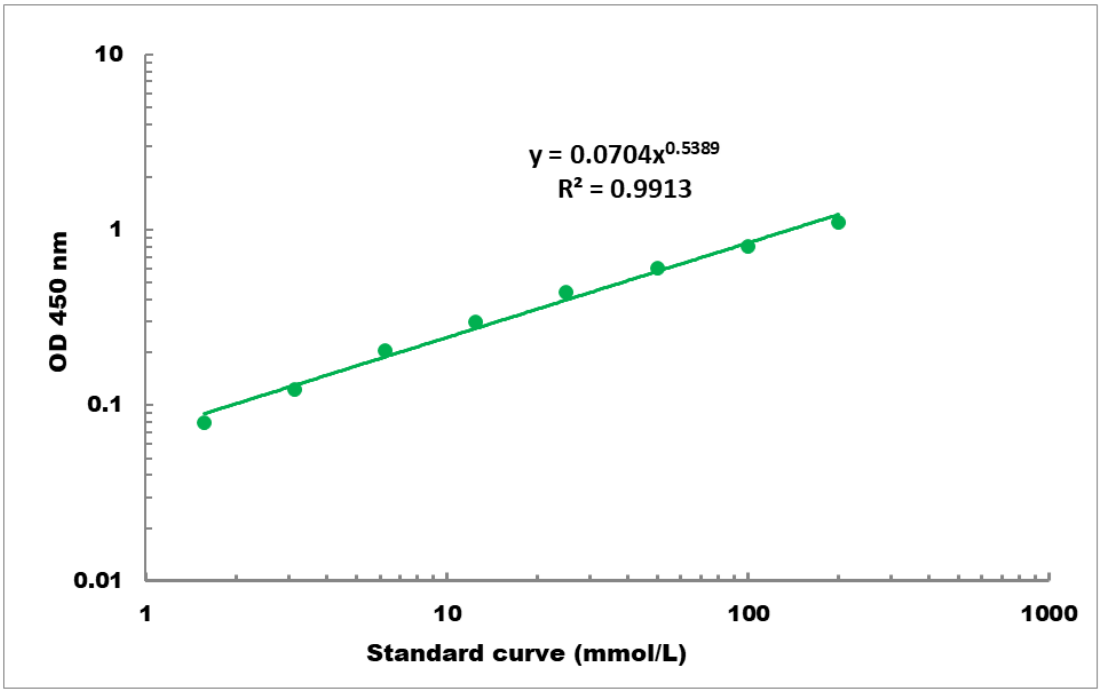
V_{Assay}: the volume of Assay Buffer, μl

$C_{Protein}$: the sample protein concentration, mg/ml

W: the weight of sample, g

VIII. 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