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Product Information

Sulfate Assay Kit

Catalog Number **MAK132**
Store at Room Temperature

TECHNICAL BULLETIN

Product 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 cerebroside sulfate, glycosaminoglycans, and heparin sulfate. Undersulfation of cartilage proteoglycans has been associated with human inherited osteochondro-dysplasia 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.

The 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 at 600 nm is proportional to sulfate level in the sample.

Components
The kit is sufficient for 200 assays in 96 well plates.

Reagent A Catalog Number MAK132A	25 mL
Reagent B Catalog Number MAK132B	2.4 g
TCA Reagent Catalog Number MAK132C	25 mL
Sulfate Standard, 60 mM Catalog Number MAK132D	1 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use clear plates.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents.

Storage/Stability
The kit is shipped and stored at room temperature.

Procedure
Standards for Turbidimetric Detection
Add 20 µL of the 60 mM Standard to 580 µL of water to prepare a 2.0 mM Standard solution. Add 0, 50, 100, and 200 µL of the 2.0 mM Standard solution into tubes. Add water to each tube to bring the volume to 200 µL, generating 0 (blank), 0.5, 1.0, and 2.0 mM standards. Transfer 200 µL of each standard into separate wells of 96 well plate.

Note: When deproteination of the sample is required (i.e., serum or plasma), the standards should also be TCA treated before they are transferred to the 96 well plate. To treat the standards, add 100 µL of TCA Reagent to 200 µL of each standard, mix, and transfer 200 µL of the resulting standard into separate wells.

Sample Preparation

Urine samples should be diluted 10-fold in water prior to assay. Fresh serum or plasma (non-hemolyzed) samples can be either assayed immediately or frozen for future tests.

If required, samples (i.e., serum and plasma) should be deproteinated as follows: mix 200 µL of sample and 100 µL of TCA Reagent in a 1.5 mL microcentrifuge tube. Spin down protein precipitates 5 minutes at 14,000 rpm on a table centrifuge. Transfer 200 µL of supernatant for assay.

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

Aliquot 200 µL of each sample into separate wells of a 96 well plate.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction

Note: The Master Reaction Mix must be prepared fresh and used within 1 hour after reconstitution.

1. Set up the Master Reaction Mix according to the scheme in Table 1. 100 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume for 10 assays
Reagent A	1 mL
Reagent B	95 mg

2. Vortex Master Reaction Mix for at least 1 minute to ensure complete dissolution of the powder and incubate the prepared Master Reaction Mix for 10 minutes before use.

3. Add 100 µL of the Master Reaction Mix to each of the appropriate wells preferably using a multi-channel pipette. Mix well using a horizontal shaker or by pipetting, and incubate the reaction at room temperature for 5 minutes.

4. Measure the optical density of the samples and standards at 600 nm for assay.

Note: This assay can be scaled up for use in cuvettes.

Results

Note: A new standard curve must be set up each time the assay is run.

Plot the optical density measured for each standard against the standard concentrations. Determine the slope using linear regression fitting.

The sulfate concentration of a sample is calculated as:

$$\text{Sulfate (mM)} = \frac{OD_{sample} - OD_{blank}}{\text{Slope}} \times n$$

where:
OD_{sample} = optical density of sample
OD_{blank} = optical density of blank (0 standard)
n = dilution factor (i.e., 10 for urine)

Note: 1 mM sulfate is equal to 9.61 mg/dL or 96.1 ppm.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For turbidimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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