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Product Information

Sulfatase Activity Assay Kit (Colorimetric)

Catalog Number **MAK276**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Sulfatases (EC 3.1.6) are a family of enzymes that catalyze the hydrolysis of sulfate ester bonds from a broad range of biological molecules, including steroids, carbohydrates, and proteins. They can be found in intracellular and extracellular spaces, and are distributed in a wide range of cells and tissues. Intracellular sulfatases are commonly found localized within the lysosome. Genetic defects in sulfatase can result in certain lysosomal storage disorders and abnormal expression can contribute to certain hormone-dependent cancers, such as breast and prostate cancer.

This Sulfatase Activity Assay Kit provides a quick and easy way to determine sulfatase activity. The kit measures the hydrolysis of a sulfate ester to 4-nitrocatechol, which can be detected at 515 nm. The kit is suitable for measuring activity of purified enzyme as well as sulfatase from biological samples. The limit of detection is below 1 mU.

Components

The kit is sufficient for 100 assays in 96 well plates.

Component	Amount
Sulfatase Assay Buffer Catalog Number MAK276A	5 mL
Sulfatase Substrate Catalog Number MAK276B	4 mL
Stop/Developing Solution Catalog Number MAK276C	10 mL
Sulfatase Catalog Number MAK276D	1 vial
4-Nitrocatechol Standard (0.5 mM) Catalog Number MAK276E	1.5 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for this assay.
- 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 all small vials prior to opening. Use ultrapure water for the preparation of reagents.

Sulfatase Assay Buffer, Sulfatase Substrate, Stop/Developing Solution, and 4-Nitrocatechol Standard – Bring to room temperature before use.

Sulfatase – Reconstitute with 20 μL of water. Aliquot and store at –20 °C. Avoid repeated freeze/thaw cycles. Stable for two months. Keep on ice during use.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended. Briefly centrifuge all small vials prior to opening.

Procedure

All samples and standards should be run in duplicate. Use ultrapure water for the preparation of samples and standards.

Sample Preparation

Homogenize cells (2×10^6 cells/mL) or tissue (50 mg/mL) in appropriate buffer (e.g., PBS) with protease inhibitors. Centrifuge at 10,000 × *g* at 4 °C for 10 minutes. Collect supernatant. Use water, 0.2% NaCl, PBS, or appropriate buffer to dissolve recombinant or purified enzyme. Add 1–10 μL of cell or tissue homogenate, or enzyme into desired wells in a 96 well plate. Prepare parallel sample well as sample background control(s). For positive control, add 2 μL of provided Sulfatase. Adjust the volume of positive control, sample background control, and sample wells to 10 μL/well with water.

Notes: For samples with unknown sulfatase activity, testing several amounts of enzyme or cell/tissue homogenate to ensure the activity is within the Standard Curve range is suggested.

To relate sulfatase activity to protein amount, measure protein concentration using a BCA Protein Assay method.

Detergents can inhibit enzymatic activity.

Standard Curve Preparation

Add 0, 20, 40, 60, 80, and 100 μL of 4-Nitrocatechol Standard into a series of wells in a 96 well plate to generate 0, 10, 20, 30, 40, and 50 nmole/well of Standard, respectively. Adjust the volume to 100 μL/well with water.

Reaction Mix

Prepare enough of the appropriate Mix for the number of wells (sample and positive control) to be analyzed. For each well, prepare 90 μL of the appropriate mix, see Table 1.

Table 1.
Preparation of Mixes

Reagent	Reaction Mix	Background Control Mix
Sulfatase Assay Buffer	50 μL	90 μL
Sulfatase Substrate	40 μL	–

Mix and add 90 μL of Reaction Mix into sample and positive control wells. and 90 μL of Background Control Mix into sample background control well. Mix well.

Measurement

Incubate plate at 37 °C for 30 minutes. After incubation, add 100 μL of Stop/Developing Solution in sample, positive control, sample background control, and Standard wells. Mix well and measure absorbance (OD 515 nm) in a microplate reader. Signal is stable for at least 30 minutes.

Results

Calculation

Subtract 0 Standard reading from all readings. Plot the 4-Nitrocatechol Standard Curve. If sample background control reading is significant, subtract background control reading from sample reading. Compare corrected OD of the sample to Standard Curve to obtain B (in nmole) of 4-Nitrocatechol generated by sulfatase during the reaction time (T = 30 minutes). To determine activity, use the following equation:

Sulfatase Activity (A) = $B/(T \times P)$
(nmole/min/mg = mU/mg)

B = amount of 4-Nitrocatechol in the sample well from Standard Curve (nmole)
P = the protein concentration (mg)
T = reaction time (minutes)

Unit Definition: One unit of sulfatase is the amount of enzyme that generates 1.0 μmole of 4-nitrocatechol per minute at pH 5 at 37 °C.

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	It is recommended to use clear plates for this assay.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used 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	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	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	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a 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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