Sulfatase Activity

Microplate Assay Kit

User Manual

Catalog # CAK1308

(Version 1.1A)

Detection and Quantification of Sulfatase Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Sulfatases (EC 3.1.6) are enzymes in the esterase class that catalyse the hydrolysis of sulfate esters from a wide 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.
Sulfatase Activity Microplate Assay Kit provides a simple and sensitive method for monitoring sulfatase activity in various samples. The kit measures the hydrolysis of a sulfate ester to 4-nitrocatechol, which can be measured at a colorimetric readout at 515 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Stop Solution	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 1 ml Reaction Buffer to dissolve before use; store at -20 °C for a month after reconstitution.

Standard: add 1 ml Reaction Buffer to dissolve before use, mix, then add 250 µl into 750 µl distilled water, the concentration will be 5 mmol/L. Store at -20 °C for a month after reconstitution.

Positive Control: add 0.1 ml distilled water to dissolve before use; store at -80 °C for a month after reconstitution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 515 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge 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 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	80 µl	80 µl	80 µl	80 µl	80 µl
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	10 µl	10 µl	--
Positive Control	--	--	--	--	10 µl
Substrate	10 µl	10 µl	10 µl	10 µl	10 µl
Standard	--	--	10 µl	--	--
Mix, put it in the oven, 37 °C for 30 minutes.					
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, record absorbance measured at 515 nm.					

Note:

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

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

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

VI. CALCULATION

Unit Definition: One unit of sulfatase activity is defined as the enzyme which generates 1 µmol of 4-nitrocatechol per minute at 37°C.

1. According to the protein concentration of sample

$$\text{Sulfatase (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample}) / T$$

$$= 0.167 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Sulfatase (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.167 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$\text{Sulfatase (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 0.167 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

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

$C_{Standard}$: the concentration of Standard, 5 mmol/L = 5 µmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

$V_{Standard}$: the volume of standard, 0.01 ml;

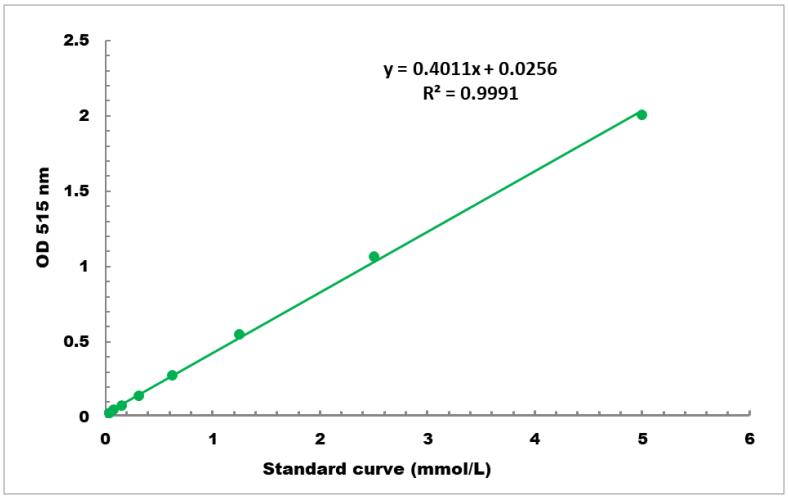
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

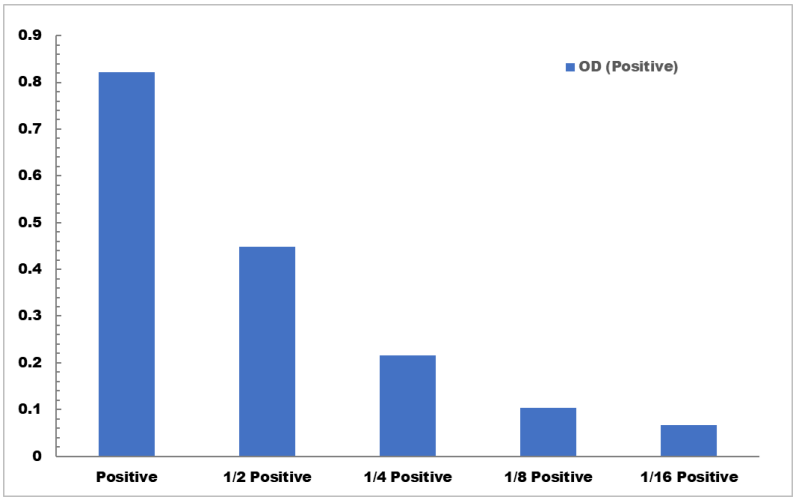
T: the reaction time, 30 min.

VII. TYPICAL DATA

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



Detection Range: 5 μmol/L - 500 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

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

IX. NOTES