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Sulfatase Activity Assay Kit (Colorimetric)

rev 09/20

(Catalog # K675-100; 100 assays; Store at -20°C)

I. Introduction:

Sulfatases (EC 3.1.6) are enzymes in the esterase class that catalyze 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. **BioVision's 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 OD 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.

II. Application:

- Measurement of sulfatase activity

III. Sample Types:

- Animal tissues such as liver, endometrium, and breast cancer
- Adherent or suspension cells
- Purified enzyme

IV. Kit Contents:

Components	K675-100	Cap Code	Part Number
Sulfatase Assay Buffer	5 ml	NM	K675-100-1
Sulfatase Substrate	4 ml	Amber/NM	K675-100-2
Stop/Developing Solution	10 ml	NM	K675-100-3
Sulfatase	1 Vial	Green	K675-100-4
4-Nitrocatechol Standard (0.5 mM)	1.5 ml	Yellow	K675-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **Sulfatase Assay Buffer, Sulfatase Substrate, Stop/Developing Solution, and 4-Nitrocatechol Standard**: Bring to room temperature (RT) before use.
- **Sulfatase:** Reconstitute with 20 µl dH_2O. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Stable for two months. Keep on ice during use.

VII. Sulfatase Activity Protocol:

1. **Sample Preparation:** Homogenize cells (2 x 10^6 cells/ml) or tissue (50 mg/ml) in appropriate buffer (e.g. PBS) with protease inhibitors (BioVision Cat. # K271 or equivalent). Centrifuge at 10,000 x g, 4°C for 10 min. Collect supernatant. Use dH_2O, 0.2% NaCl, PBS, or appropriate buffer to dissolve recombinant or purified enzyme. Add 1-10 µl 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 dH_2O.

   Notes:

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

   b. To relate sulfatase activity to protein amount, measure protein concentration using BCA Protein Assay Kit II, BioVision Cat. # K813 or equivalent.

   c. Detergents can inhibit enzymatic activity.

2. **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 nmol/well of Standard respectively. Adjust the volume to 100 µl/well with dH_2O.

3. **Reaction Mix:** Prepare enough Reaction Mix for the number of wells (sample and Positive Control) to be analyzed. For each well, prepare 90 µl Reaction Mix:

	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.

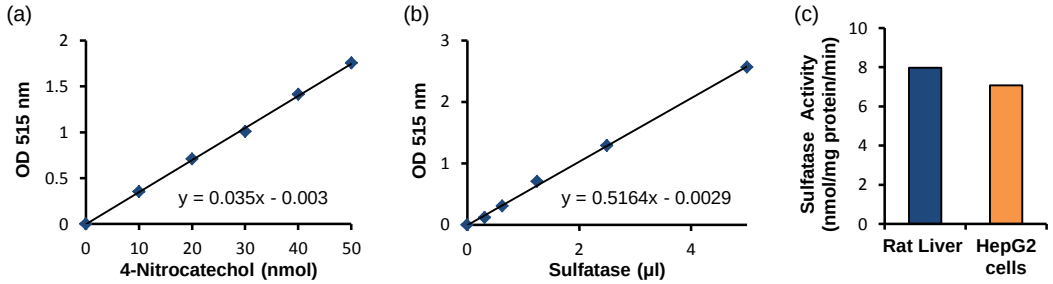
4. **Measurement:** Incubate plate at 37°C for 30 min. After incubation, add 100 µl of Stop/Developing Solution in sample, Positive Control, Sample Background Control, and Standard wells. Mix well and measure absorbance (OD 515 nm) in a microplate reader. Signal is stable for at least 30 min.

5. **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. Apply sample corrected OD to Standard Curve to get B nmol of 4-Nitrocatechol generated by Sulfatase during the reaction time (T = 30 min). To determine activity, use the following equation:

Sample Sulfatase Activity (A) = B/(T x P) = nmol/min/mg = mU/mg

Where: **B** is amount of 4-Nitrocatechol in the sample well from the Standard Curve (nmol)
P is the protein concentration (mg)
T is reaction time (min.)

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



Figures: (a). 4-Nitrocatechol Standard Curve (b). Enzyme activity measuring different amounts of the provided Sulfatase. (c). Measurement of Sulfatase Activity in HepG2 cell lysate (30 µg) and rat liver homogenate (100 µg). Assay was performed according to the kit protocol.

VIII. RELATED PRODUCTS:

BCA Protein Assay Kit II (K813)
BCA Protein Assay Kit-Reducing agent Compatible (K818)
BCA Protein Assay Kit (Test Tube)-Reducing agent Compatible (K819)
Protease Inhibitor Cocktail (K271)
Hepatic Steatosis Assay Kit (K584)
Estrogen Sulfotransferase Antibody (3829)
Phosphate-Buffered Saline (2113)
Cell Lysis Buffer (1067)
Estradiol (human) ELISA Kit (K3829)
Testosterone (human) ELISA Kit (K7417)

FOR RESEARCH USE ONLY! Not to be used on humans.