MA-0163

Sulfatase Activity Assay

(100 wells, Colorimetric, OD 515 nm, Store at -20°C)

Introduction:

Sulfatases are a group of 17 enzymes which catalyze the removal of the sulfate group from a variety of steroids and complex carbohydrates such as glycosaminoglycans. Deficiencies in sulfatase activity leads to lysosomal storage diseases where the sulfated molecules accumulate, causing functional impairment and tissue damage. The sulfatases also play a role in the regulation of hormone activity, deconjugating the sulfated forms of hormones like estrogen and DHEA which influences bioavailability and receptor binding. AkrivisBio's Sulfatase Activity Assay is a simple, sensitive means of assessing sulfatase activity in a variety of biological samples with a sensitivity below 1 mU per well.

Assay Principle:

- Sulfatase activity cleaves the sulfate group from the substrate p-nitrocatechol sulfate.
- The pH is raised to convert the p-nitrocatechol product to the phenolate ion form with strong absorbance at 515 nm.

User supplied Materials:

PBS

Assay Components:

Assay Buffer	5 ml	NM	MA-0163A
Nitrocatechol sulfate	4 ml	Amber/NM	MA-0163B
NaOH Stop Solution	10 ml	NM	MA-0163C
Sulfatase Positive Control	lyoph	Green	MA-0163D
4-Nitrocatechol Standard	1.5 ml	Yellow	MA-0163E

Storage and Handling Considerations:

Store unopened assay at -20°C. Bring all components to room temperature before using. Centrifuge small vials for a few seconds before opening.
Assay Buffer: Ready to use as supplied.
Nitrocatechol sulfate: Ready to use as supplied.
NaOH Stop Solution: Ready to use as supplied.
Sulfatase Positive Control: Reconstitute with 100 µl DI water. Aliquot into convenient portions and store at -20°C
4-Nitrocatechol Standard: Bring to room temperature (RT) before use.

Assay Protocol:

1. Standard Curve: Transfer 0 – 20 – 40 – 60 – 80 and 100 µl of Nitrocatechol Standard to a series of wells in a 96-well plate giving 0, 10, 20, 30, 40, and 50 nmol of Standard respectively. Adjust all wells to 100 µl with DI water.

2. Samples: Homogenize tissue (20 mg) or cells (2 x 10^6 cells) in PBS containing protease inhibitors (AkrivisBio PIC-0100 or equivalent). Centrifuge at 16,000 X g at 4°C for 10 minutes. Transfer the clear supernatant to a fresh tube. Transfer 1-10 µl of supernatant or purified enzyme to a 96-well plate in duplicate. For each sample, one well will be stopped at 15 minutes and the other at 30 minutes of the enzyme reaction.

3. Positive Control: Add 1-2 µl of Sulfatase Positive Control to wells in duplicate. Adjust all Sample and Positive Control wells to 10 µl with DI water.

4. Initiate Reaction: Each Sample and Positive Control well requires 90 µl of Reaction Mix. Prepare sufficient Reagent Mix for the total number of wells to be analyzed.

	Reaction Mix
Sulfatase Assay Buffer	50 µl
Sulfatase Substrate	40 µl

Add 90 µl of Reaction Mix to all Sample and Positive Control wells.

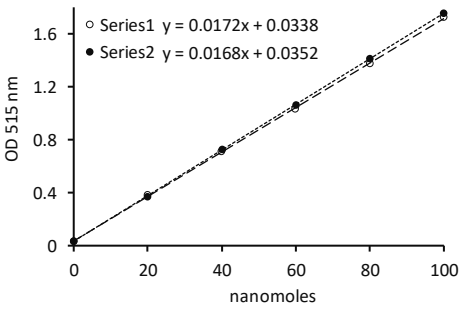
Notes:

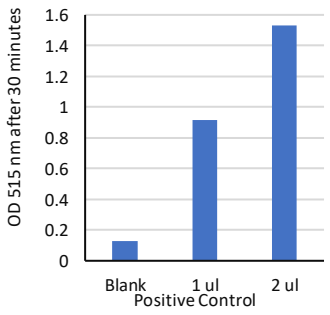
- Detergents can inhibit enzymatic activity.
- All readings (Sample and Positive Control) should be within the range of the Standard Curve. If any are outside that range, dilute appropriately and rerun.

5. Measurement: Incubate plate at 37°C for 30 min. After 15 minutes, add 100 µl of NaOH Stop Solution to one of each pair of Sample and Positive Control wells, then place back into 37°C environment. After 30 minutes, add 100 µl of NaOH Stop Solution to the remaining well of each Sample, Positive Control and Standard wells. Mix and measure absorbance at 515 nm.

6. Typical Results:

nmole Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0347	0.0357	0	0
20	0.3789	0.3740	0.3442	0.3383
40	0.7066	0.7254	0.6719	0.6897
60	1.0325	1.0656	0.9978	1.0299
80	1.3736	1.4136	1.3389	1.3779
100	1.7245	1.7550	1.6898	1.7193





7. Calculation: Subtract 0 Standard reading from all Standard, Sample and Positive Control readings. Plot the Standard Curve. Determine the slope of the Standard Curve. Divide the background corrected Sample and Positive Control readings by the slope of the Standard Curve to get nanomoles Nitrocatechol formed per incubation period (15 or 30 minutes). The 30-minute value should be close to 2X the 15 minute value. If it is substantially less, the substrate may have become limiting. Dilute the sample and rerun it. Divide the nanomoles of Nitrocatechol in the wells by the incubation period for that well to give nanomoles/minute (mU) in the well. To convert back to activity in the original sample:

- Divide the mU per well value by the volume of sample added to the well in µl = mU per µl of sample
- Multiply the mU per µl of sample X the total volume of supernatant recovered in step 2 above = Total mU of activity recovered from sample
- Divide the Total mU of activity recovered from sample by the mg tissue or # of cells used to prepare the sample = mU per mg tissue (per # of cells, etc.)

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