ab204731

Sulfatase Activity Assay Kit (Colorimetric)

Instructions for Use

For rapid, sensitive and accurate measuring of sulfatase activity.

This product is for research use only and is not intended for diagnostic use.

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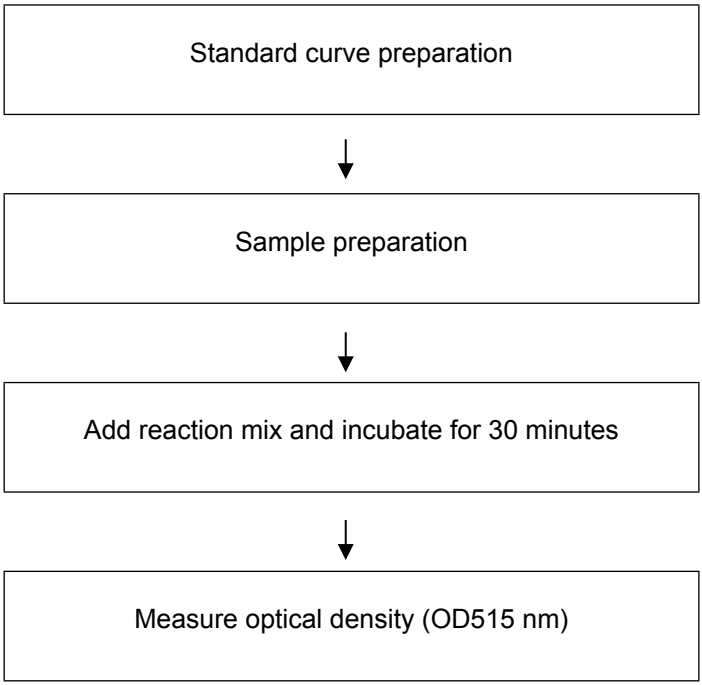
1. BACKGROUND

Sulfatase Activity Assay Kit (ab204731) 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.

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.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Sulfatase Assay Buffer	5 mL	-20°C	-20°C
Sulfatase Substrate	4 mL	-20°C	-20°C
Stop/Developing Solution	10 mL	-20°C	-20°C
Sulfatase	1 vial	-20°C	-20°C
4-Nitrocatechol Standard (0.5 mM)	1.5 mL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- NaCl (if using purified enzyme)
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD 515 nm
- 96 well plate: clear plates for colorimetric assay
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Sulfatase Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **4-Nitrocatechol Standard:**

Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays. Equilibrate to room temperature before use. Store at -20°C. Keep on ice while in use.

9.3 **Sulfatase Substrate:**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.4 **Stop/Developing Solution:**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot solution so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.5 **Sulfatase:**

Reconstitute Sulfatase in 20 µL of ddH_2O. Aliquot Sulfatase so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and cannot be stored for future use.

10.1 Using reconstituted 4-Nitrocatechol standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	ddH_2O (µL)	Final volume standard in well (µL)	End Conc. nmol in well
1	0	300	100	0
2	60	240	100	10
3	120	180	100	20
4	180	120	100	30
5	240	60	100	40
6	300	0	100	50

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells/mL).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ice cold PBS with protease inhibitors.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Incubate cells on ice for 15 – 30 minutes.

11.1.6 Centrifuge sample at 10,000 x *g* for 10 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.7 Collect supernatant and transfer to a clean tube.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 50 mg/mL).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of ice cold PBS with protease inhibitors.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Incubate sample on ice for 15 – 30 minutes.

11.2.6 Centrifuge samples 10,000 x *g* for 10 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.7 Collect supernatant and transfer to a clean tube.

11.3 **Purified enzyme:**

Dissolve purified enzyme in ddH_2O, 0.2% NaCl, PBS or appropriate buffer.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 100 µL standard dilutions.
- Sample wells = 1 – 10 µL samples (adjust volume to 10 µL/well with ddH_2O).
- Background control sample wells= 1 – 10 µL samples (adjust volume to 10 µL/well with ddH_2O).
- Positive control = 2 µL Sulfatase (adjust volume to 10 µL/well with ddH_2O).

12.2 **Reaction Mix:**

Prepare 90 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Sulfatase Assay Buffer	**50**	**90**
Sulfatase Substrate	**40**	**0**

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1).

12.3 Add 90 µL of appropriate Reaction Mix into each sample and positive control sample wells. Do not add Reaction Mix to Standard wells.

12.4 Add 90 µL of Background Reaction Mix to Background control sample wells.

12.5 Mix and incubate at 37°C for 30 minutes.

12.6 Add 100 µL of Stop/Developing Solution to all wells.

12.7 Measure output at OD 515 nm on a microplate reader.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- To relate sulfatase activity to protein amount, measure protein concentration.

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of Sulfatase.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Concentration of Sulfatase (nmol/min/mg = mU/mg) in the test samples is calculated as:

$$Sulfatase\ activity = \left(\frac{A}{B * T}\right) * D$$

Where:

A = Amount of 4-Nitrocatechol in the sample well from Standard curve (nmol).

B = Protein concentration (mg).

T = reaction time (min)

D = Sample dilution factor.

Unit Definition:

1 Unit sulfatase activity = amount of sulfatase which generates 1.0 µmol of 4-nitrocatechol per minute at pH 5 at 37°C.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

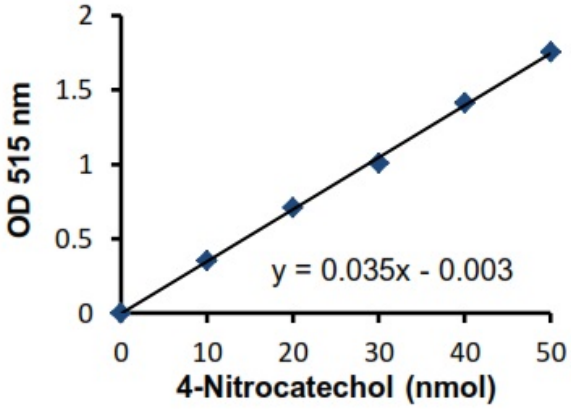


Figure 1. Typical 4-Nitrocatechol Standard calibration curve.

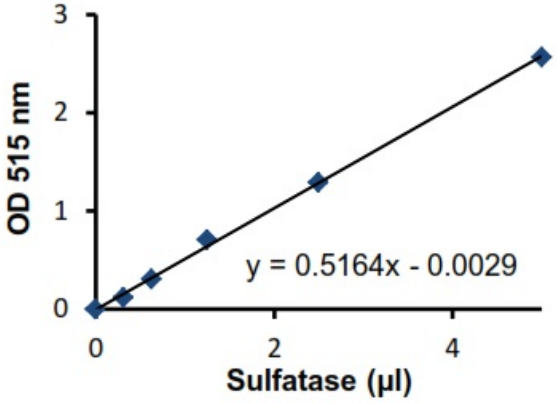


Figure 2. Enzyme activity measuring different amounts of the provided positive control Sulfatase.

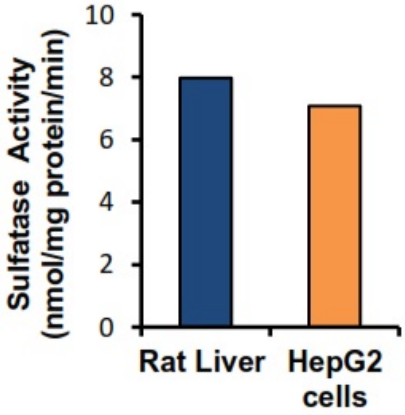


Figure 3. Measurement of Sulfatase Activity in HepG2 cell lysate (30 µg) and rat liver homogenate (100 µg).

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, substrate and Sulfatase (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (100 µL), samples (10 µL), positive control (10 µL) and background wells (10 µL).
- Prepare Sulfatase Reaction Mix (Number samples + 1).

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Sulfatase Assay Buffer	**50**	**90**
Sulfatase Substrate	**40**	**-**

- Add 90 µL of Sulfatase Reaction Mix to the positive control and sample wells. DO not add reaction mix to standard wells/
- Add 90 µL of Background Reaction Mix to the background control wells.
- Incubate plate at 37°C 30 minutes protected from light.
- Add 90 µL of Stop/Developing Solution into all wells.
- Measure plate at OD = 515 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

Which chemicals or biological materials cause interference in this assay?

RIPA buffer – contains SDS which can denature proteins and affect enzyme activity.

Detergents present in the sample (NP-40, Triton X-100) can inhibit enzymatic activity.

18. NOTES

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