ab253371
Lysosomal
Galactocerebrosidase
(GALC) Analysis Kit

View Lysosomal Galactocerebrosidase
(GALC) Analysis Kit datasheet:
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For the measurement of lysosomal galactocerebrosidase in
adherent and non-adherent cells

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

Lysosomes are acidic cytoplasmic organelles that are present in all nucleated mammalian cells. Lysosomes have been found to be involved in a variety of cellular processes including repair of the plasma membrane, defense against pathogens, cholesterol homeostasis, bone remodeling, metabolism, apoptosis, and cell signaling. Defects in lysosomal enzyme activity have been associated with a variety of neurological diseases including Parkinson’s, Tay-Sachs, Sandhoff, Wolman, Gaucher and Krabbe diseases.

Krabbe Disease is an autosomal recessive disorder that results from a deficiency in an enzyme known as galactocerebrosidase (galactosylceramidase, GALK). Defects in the gene coding for lysosomal galactocerebrosidase result in this lysosomal storage disease, affecting about 1 in 100000 live births. Krabbe Disease is also called Globoid Cell Leukodystrophy. This name derives from the characteristic pathology of Krabbe disease, where macrophages accumulate high levels of undegraded galactolipids as a result of the lack of GALK activity. These cells produce a characteristic morphology different from healthy cells, and are often termed globoid cells. The Lysosomal Galactocerebrosidase Analysis kit offers an easy to use protocol for detecting levels of lysosomal galactocerebrosidase in many cells types (adherent or nonadherent) using a lysate method and a specific lipidic fluorogenic substrate. The substrate is provided in a ready to use format in individual colored vials to allow for ease of use, stability and simple sample identification. The method also removes the need for complicated and timely substrate preparation steps.

The Lysosomal Galactocerebrosidase Analysis kit provides all the necessary reagents, buffers, and protocols for up to 30 lysosomal galactocerebrosidase assays.
2. Materials Supplied and Storage

Store kit immediately on receipt and check below for storage for individual components. Kit can be stored for 6 months from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate Reagent</td>
<td>30 units</td>
<td>-20°C (Light Sensitive)</td>
</tr>
<tr>
<td>Lysis Buffer A</td>
<td>20 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Lysis Buffer B</td>
<td>20 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Reaction Buffer</td>
<td>5 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Stop Buffer</td>
<td>20 mL</td>
<td>4°C</td>
</tr>
</tbody>
</table>

Fluorescent reagents and fluorogenic substrates should be handled with care, kept cold when not in use, and stored at 20°C. In case of contact with skin or eyes wash thoroughly with soap and cold water. High background fluorescence readings for blank samples will indicate decomposition. Please contact us for information on use or licensing.
3. Materials Required, Not Supplied

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

- UV-VIS Spectrometer or Plate reader.
- Ultrasonifier
- BCA Assay
- Absolute ethanol

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.
5. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in triplicate.

5.1 Harvest the cells to be assayed by centrifugation (800 rpm, 5 minutes) or at an appropriate speed and time for the cell line or cell type under analysis.

\[\textbf{\Delta \text{Note}}: \text{recommended cell number } > 1 \times 10^6 \text{ per sample.}\]

5.2 Carefully remove the culture supernatant by aspiration and resuspend the cell pellet in 0.5mL of Lysis Buffer A.

5.3 Lyse the cells using 2 x 30 seconds, 20% duty cycle bursts of ultrasound from an ultrasonifier fitted with a microtip. Alternately, the cells can be lysed using one of the methods described in the References below.

5.4 Centrifuge the lysate at 27,000 x g for 30 minutes, aspirate and retain the supernatant.

5.5 Resuspend the pellet in 0.5mL of Lysis Buffer B.

5.6 Perform further lysis on the pellet using 2 x 30 seconds, 20% duty cycle bursts of ultrasound from an ultrasonifier fitted with a microtip.

5.7 Centrifuge the second lysate at 27,000 x g for 30 minutes, aspirate and combine the supernatant with that from Lysis Buffer A.

5.8 Calculate total protein concentration of the combined lysates using a BCA assay or another equivalent method.

5.9 Determine volume of lysate required to give 50 µg of protein.

5.10 Calculate the volume of Reaction Buffer required to bring total reaction volume (Reaction Buffer + lysate) to 100 µL.

5.11 Add the calculated volume of Reaction Buffer (from step 5.10) to one Substrate Reagent vial and vortex mix this substrate reagent solution for 2-3 minutes to ensure complete reconstitution of the substrate (dried substrate is no longer visible on the sides of the vial).

5.12 Add the lysate volume from Step 5.9 to the Substrate Reagent Vial from Step 5.11 (100 µL total volume) and incubate at 37°C for 2 hours.

5.13 A blank containing Reaction Buffer with an equivalent volume of 1:1 Lysis Buffer A: Lysis Buffer B should also be prepared and incubated in one Substrate Reagent vial as above.
5.14 Prepare enough Stop Buffer Solution for all samples by diluting included Stop Buffer 1:1 with absolute ethanol. 
\textbf{\Delta Note:} You will need 1 mL of diluted Stop Buffer Solution for each sample from above.

5.15 Add the contents of the reaction mixture vial to 1mL of diluted Stop Buffer Solution. Add the contents of the blank vial to 1mL of diluted Stop Buffer Solution. Read the fluorescence of each at 365nm/454nm using a UV-VIS Spectrometer or Plate reader.

5.16 Subtract values obtained from blanks from all sample readings. 
\textbf{\Delta Note:} It is recommended that all samples be analyzed in at least triplicate and values averaged.
6. Typical Data

Data provided for demonstration purposes only.

Figure 1. Leukocytes derived from a healthy individual and from a Krabbe patient were assayed using the protocol above. Fluorescence was recorded using a Tecan Infinite M200 Pro plate reader, blank readings were subtracted from samples and an average of 6 wells presented.
7. References:


8. Notes
Technical Support

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