

Product Data Sheet

Product Name: 2-NBDG
Cat. No.: GC10289

Chemical Properties

Cas. No. 186689-07-6

Chemical Name (3R,4R,5S,6R)-6-(hydroxymethyl)-3-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)tetrahydro-2H-pyran-2,4,5-triol

SMILES OC[C@](O1)([H])[C@](O)([H])[C@@](O)([H])[C@](NC2=CC=C(N(=O)=O)C3=NON=C23)([H])C1([H])O

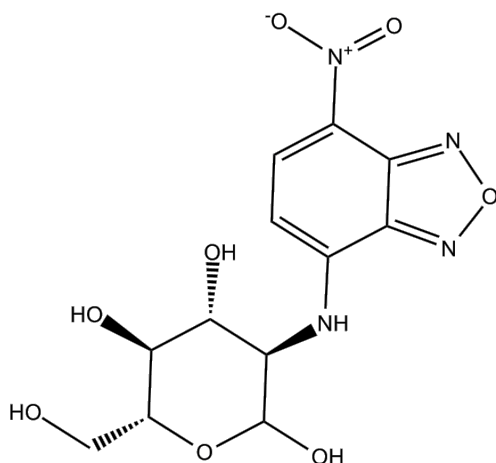
Formula $C_{12}H_{14}N_4O_8$ M.Wt 342.26

Solubility ≥ 17.1 mg/mL in Water with ultrasonic Storage -20°C , protect from light

General tips For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Procedure for 2-NBDG uptake assay for MEFs [1]

1. Mouse embryonic fibroblasts (MEFs) are isolated from the embryos of C57BL/6 WT mouse (13.5 days).
2. Culture the MEF cells until reaching 80-90% confluence in 10 cm Petri dishes with DMEM growth

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medium in a humidified cell culture incubator (37 °C, 5% CO₂).

Note: Don't use MEFs beyond passage 3. MEFs usually become senescent at about passage 4 to 5.

3. Remove culture medium and wash cells one time with 10 ml 1x PBS.

4. Trypsinize cells using 4 ml of 0.05% trypsin-EDTA for 3 min at 37 °C.

Note: Use room temperature or pre-warmed 1x PBS from Step A3 to Step A9. Using chilled 1x PBS after Step A9.

5. Transfer cells to 15 ml polystyrene centrifuge tubes.

6. Harvest cells at 200 x g for 5 min by centrifugation.

7. Wash pelleted cells one time with 5 ml 1x PBS.

8. Count cells using a hemocytometer chamber.

9. Incubate 1 x 10⁶ MEF cells in a 37 °C water bath for 2 h with 1 ml of PBS containing 100 µM 2-NBDG. Incubate the same number of MEFs in the water bath with 1 ml PBS without 2-NBDG as a negative control.

10. Pellet the cells at 200 x g for 5 min by centrifugation. After washing the cells with chilled 1x PBS, the cells are pelleted at 200 x g for 5 min by centrifugation.

11. Resuspend cells in 0.5 ml of ice-cold 1x PBS with 2% FBS.

Note: Always keep cells on ice after this step.

12. Filter cells through a 35 µm nylon mesh (**the cell-strainer cap of the 5 ml round-bottom polystyrene tubes**) to obtain a uniform single-cell suspension in a 5 ml tube.

13. Keep the samples on ice until analysis on a flow cytometer.

14. Perform flow cytometric analysis. Acquire 10,000 single-cell events per reaction.

15. Analyze fluorescence intensity

Procedure for 2-NBDG uptake assay for breast cancer cells [1]

Using the same procedure as MEFs' uptake assay except incubating 1 x 10⁶ MCF7 cells in a 37 °C water bath only for 30 min (instead of two hours for MEFs) with 1 ml of PBS containing 100 µM 2-NBDG.

10 mM stock of 2-NBDG: Dissolve 5 mg 2-NBDG in 1.46 ml PBS. Store at -20 °C in the dark

This protocol only provides a guideline, and should be modified according to your specific needs

References:

[1]. Dong, S., Baranwal, S., Garcia, A., Serrano-Gomez, S. J., Eastlack, S., Iwakuma, T., Mercante, D., Mauvais-Jarvis, F. and Alahari, S. K. (2017). Nischarin inhibition alters energy metabolism by activating AMP-activated protein kinase. J Biol Chem 292(41): 16833-16846.

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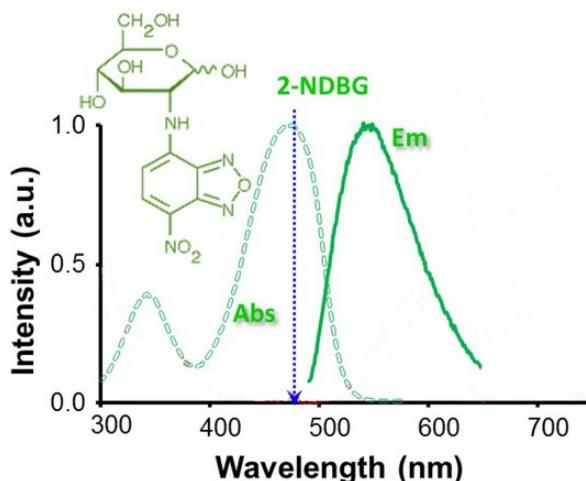
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Background

2-NBDG is a fluorescence-labeled 2-deoxy-glucose analog useful as a tracer for evaluation of cellular glucose metabolism (Ex/Em: 475/550 nm).



Glucose is a necessary source of energy for sustaining cell activities and homeostasis in tissues. Glucose metabolism is an important target in many diseases and changed with the pathological condition, therefore, evaluation of glucose metabolism can be a significant indication in disease progressions.

2 NBDG can be used in many kinds of cells in vitro, such as HepG2 human hepatocarcinoma cells, L6 rat skeletal muscle cells, MCF-7 breast cancer epithelial cells and astrocytes, it is also used in disease models, epilepsy rat, hyperglycemia, diabetes or mouse xenograft model of cancer.

2-NBDG enters cells through glucose transporters and is subsequently phosphorylated by hexokinase and trapped inside cells. Flow cytometric detection of fluorescence produced by cells can be performed to examine 2 NBDG uptake into living cells, and the intracellular concentration of transported 2-NBDG can be measured with a fluorescence microplate assay. 2 NBDG can be detected with a fluorescence imaging microscopy or CCD camera simply as well.

2-NBDG is a fluorescently labeled glucose tracer that is transported into cells by the same glucose transporter (GLUT) as glucose. Once 2-NBDG is taken up by cells, it is phosphorylated at the C-6 position to give 2-NBDG-6-phosphate, which is well retained in the cell. Compared to other glucose tracers such as 2-DG or FDG, 2-NBDG enables in situ measurement of 2-NBDG with high temporal and spatial resolution at the single-cell level. (suitable for fluorescence microscopy and flow cytometry)

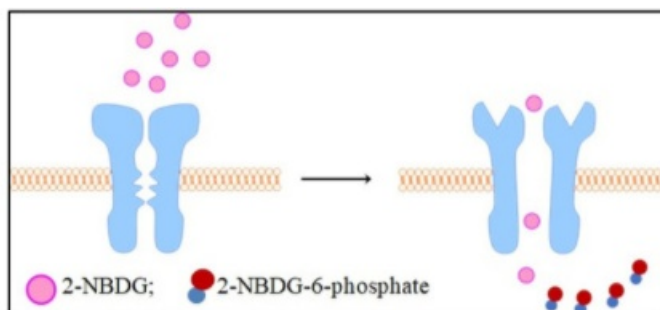
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detection)



Rationale for 2-NBDG glucose uptake assay in cells: Once 2 NBDG is taken up by cells, it is phosphorylated at the C-6 position to generate 2-NBDG-6-phosphate in 2 NBDG metabolism, which is well retained in the cell, the fluorescence intensity is proportional to the cellular glucose uptake activity.

References:

- [1]. Zou C, Wang Y, Shen Z. 2-NBDG as a fluorescent indicator for direct glucose uptake measurement[J]. Journal of biochemical and biophysical methods, 2005, 64(3): 207-215.
- [2]. O'Neil R G, Wu L, Mullani N. Uptake of a fluorescent deoxyglucose analog (2-NBDG) in tumor cells[J]. Molecular Imaging and Biology, 2005, 7(6): 388-392.
- [3]. Tsytsarev V, Maslov K I, Yao J, et al. In vivo imaging of epileptic activity using 2-NBDG, a fluorescent deoxyglucose analog[J]. Journal of neuroscience methods, 2012, 203(1): 136-140.
- [4]. Yan Chen, Junjian Zhang, Xiang-yang Zhang, 2-NBDG as a Marker for Detecting Glucose Uptake in Reactive Astrocytes Exposed to Oxygen-Glucose Deprivation In Vitro. J Mol Neurosci (2015) 55:126–130.
- [5]. Tsytsarev V, Maslov K I, Yao J, et al. In vivo imaging of epileptic activity using 2-NBDG, a fluorescent deoxyglucose analog[J]. Journal of neuroscience methods, 2012, 203(1): 136-140.

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