# **Product** Data Sheet

# Fluo-4 AM

Cat. No.:HY-101896CAS No.:273221-67-3Molecular Formula: $C_{51}H_{50}F_2N_2O_{23}$ Molecular Weight:1096.94

Target: Fluorescent Dye

Pathway: Others

Storage: -20°C, sealed storage, away from moisture and light

\* The compound is unstable in solutions, freshly prepared is recommended.

#### **BIOLOGICAL ACTIVITY**

#### Description

Fluo-4 AM is a cell-permeable Ca<sup>2+</sup> indicator<sup>[1]</sup>.

#### In Vitro

Fluo-4 AM is a fluorescent dye ( $\lambda_{ex}$ =494 nm,  $\lambda_{em}$ =516 nm). Preloaded with Fuo-4 AM, a very bright fluorescence image is observed. In a parallel experiment with fluo-3 AM-loaded cells, the resulting fluorescence image, although clearly discernable in this case, is less bright<sup>[1]</sup>.?Guidelines (Following is our recommended protocol. This protocol only provides a guideline, and should be modified according to your specific needs).

?1.?Count the cells and take 106 cells from each sample (control and experiment/s).

?2.?Collect the cells (5 min, 3000×g, 4  $^{\circ}\text{C})$  and wash once in PBS.

?3.?Resuspend the cells in 0.5-ml PBS and add 0.5  $\mu$ l of Fluo-4-AM (1 mM stock) to a final concentration of 1  $\mu$ M. Incubate at 27 °C for 1 b

?4.?Wash the cells three times (2 min, 3000×g) with PBS and finally resuspend in 1-ml PBS. Separate into 2 flow cytometry tubes—0.5 ml in each.

?5.?Evaluate the staining by flow cytometry and analyze the data by a software such as CellQuest software.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## **PROTOCOL**

Cell Assay [1]

For measuring fluorescence from cells in suspension, dilutions to 2 to  $3\times10^6$  cells are made, from cultures of rat basophilic leukemia (RBL) cells. Cells are incubated in suspension in 1  $\mu$ M dye (including Fluo-4 AM) for 30 min at 37°C. Cell suspensions are then transferred to cuvets for measurements of fluorescence emission intensity by spectrofluorometer<sup>[1]</sup>.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## **CUSTOMER VALIDATION**

- Bioact Mater. 2022 May 21;20:81-92.
- ISME J. 2023 Nov 10.
- J Adv Res. 2023 Sep 13;S2090-1232(23)00257-6.
- Nano Res. 2022;15(10):9286-9297.

REFERENCES

[1]. Gee KR, et al. Chemical and physiological characterization of fluo-4 Ca(2+)-indicator dyes. Cell Calcium. 2000 Feb;27(2):97-106.

[2]. Fluo-4.

• Acta Biomater. 2023 Apr 20;S1742-7061(23)00219-2.

Caution: Product has not been fully validated for medical applications. For research use only.

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