（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F103

Specification：96T

Measuring instrument：Fluorescence Microplate reader, Fluorescence microscope, Flow Cytometry

Elabscience® Cell Hydrogen Sulfide (H_2S) Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Intended use

This kit can be used to measure the level of hydrogen sulfide (H_2S) in cell samples.

Detection principle

Hydrogen Sulfide (H_2S), as a key signal transduction gas molecule in cells and organisms, is involved in numerous physiological and pathological processes such as vascular relaxation and nerve transmission. The detection of intracellular H_2S can deeply explore its specific mechanism and role in cellular signal transduction. Given that abnormal intracellular H_2S concentration is associated with various diseases such as diabetes and liver cirrhosis, the detection of intracellular H_2S can play an auxiliary role in the early diagnosis of diseases, condition monitoring, and evaluation of treatment effects.

The detection principle of this kit: the probe produce green fluorescence with H_2S after entering the cell. The level of H_2S in cells can be detected by measuring the fluorescence intensity.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL ×2 vials	-20℃, 12 months, shading light
Reagent 2	Probe	0.44 mL ×1 vial	-20℃, 12 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescence microplate reader, Fluorescence microscope, Flow cytometry

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to 25℃ before use. Store the aliquoted probe at -20 ℃ protected from light, and avoid repeated freeze/thaw cycles is advised.

② The preparation of probe working solution:

Dilute probe with buffer solution to 50-200 times (the initial recommended dilution factor is 50 times). The probe working solution should be prepared on spot protected from light and used up within 1 day.

④ The amount of probe working solution in different petri dishes:

Cell type	**Adherent cells**				**Suspension cells**
Well	6-well plate	24-well plate	96-well plate	35 mm petri dish	2 mL EP tube
The volume of probe working solution	1.5 mL/well	0.3 mL/well	0.1 mL/well	1.5 mL/well	0.2 mL/tube

Operating steps

Instrument parameter	
Fluorescence microplate reader	Ex/Em = 490 nm/528 nm
Flow cytometry	FITC
Fluorescence microscope	Ex/Em = 490 nm/528 nm

Note: The sensitivity of different detection instruments is different, combined with the instrument detection signal, through the pre-experiment to adjust the dilution factor of the probe working solution.

1. Suspension cell:

① For 2 mL EP tube, the number of cells per tube is at least 1×10^5. Wash the cells with PBS (0.01 M, pH 7.4) for twice. Centrifuge at 500×g for 3 min at room temperature.

② The negative control group was recommended for each experiment. For negative control group, add 0.2 mL of buffer solution to resuspension cells. For sample group, add 0.2 mL of probe working solution to resuspension cells. Please adjust the concentration of working solution and incubation time according to the actual experimental situation.

③ Incubate at 37℃ for 30~60 min protected from light. Please adjust the concentration of working solution and incubation time according to the actual experimental situation.

④ Wash the cells with PBS (0.01 M, pH 7.4) for twice. Centrifuge at 500×g for 3 min at room temperature.

⑤ Fluorescence microplate reader: Add 0.1 mL of cell suspension to 96 wells black microplate to detect the fluorescence value.
Flow cytometry: Direct detection.
Fluorescence microscope: Made slides of cells and take photos for observation.

2. Adherent cell:

① For 24-well plate, the number of cells inoculated in each well should be at least 5×10^4. Remove medium. Wash the cells with PBS (0.01 M, pH 7.4) for twice.

② The negative control group was recommended for each experiment. For negative control group, add 0.3 mL of buffer solution. For sample group, add 0.3 mL of probe working solution.

③ Incubate at 37℃ for 30~60 min protected from light. Please adjust the concentration of working solution and incubation time according to the actual experimental situation.

④ Wash the cells with PBS (0.01 M, pH 7.4) for twice.

⑤ Fluorescence microplate reader: After cell digestion, add PBS (0.01 M, pH 7.4) to resuspension cells. Add 0.1 mL of cell suspension to 96 wells black microplate to detect the fluorescence value.

Flow cytometry: After cell digestion, add PBS (0.01 M, pH 7.4) to resuspension cells.

Fluorescence microscope: Take photos for observation.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.