BT LAB
Bioassay Technology Laboratory

Optimize Your Research

Reactive Oxygen Species Assay Kit

USER INSTRUCTION

Cat.No SH0403

Method: Chemical fluorometric method

Size: 100T - 500T

***This product is for research use only, not for use in diagnosis procedures. It's highly recommend to read this instruction entirely before use.**

Detection significance

Reactive oxygen species (ROS) include the super oxygen free radical, hydrogen peroxide and its downstream product (peroxide and hydroxide) etc. ROS is involved in cell growth, proliferation, differentiation of development, aging and apoptosis as well as many physiological and disease process. The DCFH-DA (2, 7-dichlorofuorescin diacetate) is the most commonly used and most sensitive intracellular reactive oxygen detection probe. DCFH-DA has no fluorescence, it is hydrolyzed into DCFH (dichlorofluorescin) by ester hydrolysis enzyme after entering cells. DCFH can be oxidized to strong green fluorescence material DCF (dichlorofluorescein) that cannot pass through cell membrane when ROS exists. The excitation wavelength and the maximum emission wavelength of DCF is 502nm and 530nm respectively. The fluorescence intensity is proportional to the level of reactive oxygen species in cells.

Work wavelength: optimal excitation wavelength is 500 nm, 485 nm (500±15 nm), optimal emission wavelength is 525 nm (530±20 nm). It can also be detected according to the fluorescence detection conditions of FITC.

Kit components

Item	Component	Specifications	Storage
Reagent 1	10 mM DCFH-DA in DMSO	0.1 mL	-20 ℃
Reagent 2	ROS hydrogen donor	1 mL	4-8 ℃

Reagent preparation (for cells)

1. DCFH-DA can be diluted in culture medium or buffer. The color of serum or medium does not affect DCFH-DA and intracellular fluorescence, but may affect the fluorescence detection of fluorescent microscopy, fluorescence spectrophotometer, and fluorescence microplate or flow cytometry instrument. DCFH-DA can be diluted with non-phenol red culture medium or appropriate buffer such as PBS. It depends on the type of fluorescent detection devices.

2. The timing of adding DCFH-DA or incubation time depends on whether the intracellular reactive oxygen species can be detected successfully. DCFH-DA can be added in advance or at the same time if the drug treatment time is short (< 2 h) or the predicted ROS is weak. Conversely, DCFH-DA can be added later if the drug treatment time is long (> 6 h) or predicted ROS is strong.
3. The ROS hydrogen donor contains high purity 12 mM H2O2 which is a kind of ROS in cells. And other types of ROS will be produced in metabolism process which can make the DCFH-DA be oxidized into DCF and present strong green fluorescence. Therefore, users can use the reagent as positive control to detect the experimental system or equipment, so as to preliminary observe the general characteristics of the fluorescence produced by intracellular ROS. It is recommended that cellular working concentration of the reagent is 20~100 M or lower (if more than 200 μM, cytotoxic will appears. If the user is familiar with ROS fluorescent or positive control for the experiment is not necessary, such as fluorescent microscope detection, it does not need to add this reagent.)

Operation steps

1. Detection of culture cell sample (can be observed with flow cytometry, fluorescence microplate, fluorescence photometer or fluorescence microscope)

(1)Add the fluorescent probe:

a. Add DCFH-DA into the culture medium. It is recommend that initial work concentration should be 10 μM. The DCFH-DA working concentration can be 100 nM~20 μM for different cells and treatment. Pre-experiment is suggested to determine the appropriate concentration. The total dilution ratio should be more than 1:500-1:1000 in order to avoid effects of DMSO on cells. DMSO should be set as solution control.

b. Incubate at 37 ℃ for 30 min ~ few hours, generally 30~60 min. The incubation time is related to cell types, stimulation conditions, and DCFH-DA concentration.

c. Cell collection:

Suspension cells: centrifuge the sample at 1000 g for 5~10 min and wash with PBS for 1~2 times. Centrifuge and collect the cell precipitation for fluorescence detection.

Adherent cells: digest the cells with 0.25% trypsin, add medium to terminate the digestion, thus to prepare the cell suspension. Centrifuge at 1000 g for 5~10 min and collect cells, then wash with PBS for 1~2 times. Centrifuge and collect cell precipitation for fluorescence detection.

(2) Fluorescence detection:

a. Re-suspend collected cells with PBS or non-phenol red culture medium for detection. (Re-suspend with PBS is recommended)

b. Wavelength: the optimal excitation wavelength is 500 nm, 485 nm (500±15 nm), optimal emission wavelength is 525 nm (530 ±20 nm). It can also be detected according to the fluorescence detection conditions of FITC.

Notes: the density of re-suspension cell is determined by cell fluorescence intensity. If fluorescence is strong (weak), then decrease (increase) the cell density, but cell density of all samples should be consistent.

2. Detection of tissue sample (can be observed with flow cytometry fluorescence microplate, fluorospectrophotometer)

(1)Preparation of single cell suspension:

Method 1: using the single cell suspension instrument.

Method 2: enzyme digestion.

a. Take the tissue into pre-cooled tissue culture medium or PBS immediately and clean the blood and other contaminants. Remove the massive composition, fiber, fat, and blood vessels (except for specialized cells).

b. Cut the tissue into about 1 mm3 pieces with the ophthalmic scissors, then put these pieces to pre-cooled tissue culture medium or PBS to remove the cell debris.

c. Add an appropriate amount of enzyme digestion, incubate in 37 ℃ water bath for 20~30 min and oscillate the mixture intermittently.

d. Stop the digestion with cooled culture solution or PBS. Filter the mixture to remove the tissuemassive component with nylon mesh and collect the cells. Centrifuge at 500 g for 10 min and discard the supernatant, then wash with PBS for 1~2 times. Re-suspend to prepare the single cell suspension solution. The cell amount should be no less than 106.

Method 3: mechanical method.

a. The pretreatment is the same as step 1 and step 2 in the enzyme digestion method.

b. Tight the nylon mesh on a small beaker, then place the tissue pieces on the mesh and gently rub the tissue with ophthalmic scissor or erasing knife. Wash the tissue with PBS at the same time.

c. Collect the cell suspension and centrifuge at 500 g for 10 min. Then discard the supernatant and wash with PBS for 1~2 times. Re-suspend to prepare the single cell suspension solution. The cell amount should be no less than 106.

(2)Add the fluorescent probe:

a. Add DCFH-DA into the culture medium. It is recommend that initial work concentration should be 10 μM. The DCFH-DA working concentration can be 100 nM~20 μM for different cells and treatment. Pre-experiment is suggested to determine the appropriate concentration. The total dilution ratio should be more than 1:500-1:1000 in order to avoid effects of DMSO on cells. DMSO should be set as solution control.

b. Incubate at 37 ℃ for 30 min ~ few hours, generally 30~60 min. The incubation time is related

c. to cell types, stimulation conditions, and DCFH-DA concentration.

d. Collect the incubated (labelled with probe) single cell suspension, centrifuge at 1000 g for 5~10 min to collect cells. Wash with PBS for 1~2 times. Centrifuge and collect the cell precipitation for fluorescence detection.

(3)Fluorescence detection:

a. Re-suspend collected cell precipitation with PBS for detection.

b. Wavelength: the optimal excitation wavelength is 500 nm, 485 nm (500±15 nm), optimal emission wavelength is 525 nm (530 ±20 nm). It can also be detected according to the fluorescence detection conditions of FITC.

Notes:

1. The density of re-suspension cell is determined by cell fluorescence intensity. If fluorescence is strong (weak), then decrease (increase) the cell density, but cell density of all samples should be consistent.

Take some single cell suspension and treat with ultrasonication or homogenate for protein determination, which can be used for calculation of results and represented as Fluorescence value/mg protein.