



ISO 13485:2016 ISO 9001:2015

Ver.250201

# Reactive Oxygen Species Detection Kit

CA1410 (100 Tests)

**FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS**

## Product Description

Reactive Oxygen Species (ROS) Assay Kit detects reactive oxygen species using fluorescent probe DCFH-DA. DCFH-DA itself has no fluorescence and can freely pass through the cell membrane. After entering the cell, it is hydrolyzed by intracellular esterase into a non-fluorescent molecule. ROS in the cell oxidises this into 2', 7'-dichlorofluorescein (DCF). DCF is highly fluorescent and is detected by fluorescence spectroscopy with excitation / emission at 485 nm / 535 nm.

## Kit components

Reagent	Volume	Storage
DCFH-DA (10 mM)	0.1mL ×1	-20°C
Positive Control	1mL ×1	4°C

## Instructions for use:

For cells with short stimulation time (usually within 2 hours), load probes first, and then stimulate cells with reactive oxygen species positive control or drugs of interest. For cells with long cell stimulation time (usually more than 6 hours), first stimulate cells with reactive oxygen species positive control or drugs of interest, and then load probe.

Positive control working solution: Take 1µL Positive control and mix it with 17mL serum free medium. Use this to treat the cells. Do not store for later use. The final volume of the working solution can be changed by altering the volumes of positive control and serum free medium but keeping the ratio constant.

### *Protocol for adherent cultured cells*

- Dilute DCFH-DA 1000 times in serum-free medium to a final concentration of 10µmol/L.
- Remove the cell culture medium and add an appropriate volume of diluted DCFH-DA.
- The volume to be added should be sufficient to cover the cells.  
*Usually, 1mL of diluted DCFH-DA is added to one well of a six-well plate.*
- Incubate in a 37°C cell incubator for 20 minutes.
- Wash the cells 3 times with serum-free cell culture medium to remove the excess DCFH-DA.
- Usually ROS levels in the positive control treated cells will significantly increase after stimulating cells for 20-30 minutes.

### *Protocol for cell suspension*

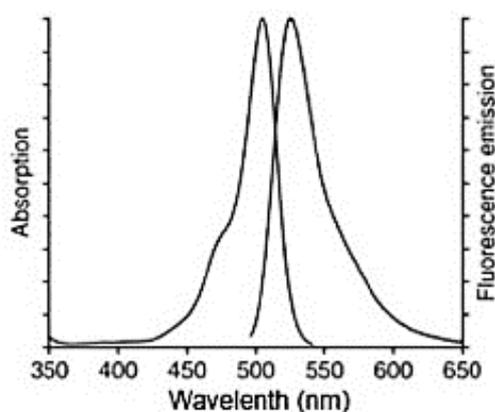
- Dilute DCFH-DA 1000 times in serum-free medium to a final concentration of 10µmol/L.
- Collect the cells and suspended in diluted DCFH-DA at a cell concentration of 1 million to 20 million/ml, and incubated in a 37°C cell incubator for 20 minutes.  
*Mix by inversion every 3-5 minutes to allow adequate contact between the probe and the cells.*
- Washed three times with serum-free cell culture medium to sufficiently remove DCFH-DA that did not enter the cells.
- Directly stimulate the cells with reactive oxygen species positive control or drugs of interest, or divide the cells into several parts and stimulate the cells.
- Usually ROS positive controls can significantly increase ROS levels after stimulating cells for 20-30 minutes.

### ***Detection***

Samples can be directly observed with a laser confocal microscope, or cells can be collected and detected with a fluorescence spectrophotometer, a fluorescence microplate reader, or a flow cytometer.

### ***Parameter setting***

Use 488nm excitation wavelength and 525nm emission wavelength to detect the intensity of fluorescence before and after stimulation in real time or time point by time. The fluorescence spectrum of DCF is very similar to that of FITC, and DCF can be detected with the parameter settings of FITC. The excitation and emission spectra of DCF are shown in the figure below.



### **Note**

- Positive controls can be used at a ratio of 1:1000. For example, a total of 1 ml of cells loaded with probes can be stimulated by adding 1 microliter of positive control. A very significant increase in reactive oxygen species levels is usually observed within 20-30 minutes of stimulation. For different cells, the effect of reactive oxygen species positive control may be quite different. If no increase in reactive oxygen species is observed within 30 minutes after stimulation, the concentration of reactive oxygen species positive control can be appropriately increased. If the active oxygen rises too fast, the concentration of the active oxygen positive control can be appropriately reduced.
- In addition, for some cells, if the negative control cells without stimulation are found to have strong fluorescence, DCFH-DA can be diluted 1:2000-1:5000.
- The probe loading time can also be adjusted appropriately within 15-60 minutes according to the situation.