# TECHNICAL MANUAL

# Reactive Oxygen Species (ROS) Fluorometric Assay Kit

- **SKU CODE:** MAES0112
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

# 1. Intended use

This kit can be used to measure reactive oxygen species (ROS) in fresh tissue and living cell samples.

# 2. Detection principle

DCFH-DA (2,7-dichlorofluorescin diacetate) is a non-fluorescent probe that can freely cross cell membranes. After entering the cell, it is hydrolyzed by intracellular esterase to form DCFH (dichlorofluorescin). In the presence of reactive oxygen species (ROS), DCFH is oxidized to DCF (dichlorofluorescein), which is a strongly fluorescent green substance that cannot penetrate the cell membrane. DCF has a maximum peak near the excitation wavelength of 502 nm and the emission wavelength of 525 nm, and its intensity is proportional to the level of intracellular reactive oxygen species.

# 3. Kit components & storage

| Item | Component | Size (96 T) | Storage |
|---|---|---|---|
| Reagent 1 | 10 mmol/L DCFH-DA | 0.1 mL x 1 vial | -20 °C, 12 months, shading light |
| Reagent 2 | Positive Control | 1 mL x 1 vial | -20 °C, 12 months, shading light |
| | Black Microplate | 96 wells x 2 | No requirement |
| | Plate Sealer | 4 pieces | |

# 4. Materials prepared by users

**Instruments:**

Fluorescence Microplate reader (Ex/Em=488 nm/525 nm), Fluorescence Microscope (Ex/Em=488 nm/525 nm), Flow Cytometry (Ex/Em=488 nm/525 nm), Vortex mixer, Micropipettor, Water bath, Incubator, Centrifuge

**Reagents:**

Double distilled water, PBS (0.01 M, pH 7.4), serum-free medium

# 5. Reagent preparation

1. **Preparation of DCFH-DA working solution:** Dilute the 10 mmol/L DCFH-DA with serum-free medium. The recommended working concentration is 0.1-20 μM. (Note: DMSO is harmful to cells, so the dilution ratio must be more than 1:500.) The DCFH-DA working solution should be prepared immediately before use.
2. **Preparation of positive control working solution:** Dilute the positive control (containing 10 mM TBHP) with serum-free medium. The recommended working concentration of TBHP is 50-250 μM. The positive control working solution should be prepared immediately before use.

# 6. The key points of the assay

1. After probe incubation, it is important to thoroughly wash away any residual probes that have not entered the cells, otherwise the background will be higher.
2. Avoid repeated freezing and thawing of DCFH-DA.
3. Minimize detection time to reduce experimental error.
4. Set a positive control (positive control working solution) and a negative control (cells only without 10 mmol/L DCFH-DA working solution).

# 7. Operating steps

Detection of culture cell sample

1. Add the fluorescent probe:

① Add DCFH-DA working solution to the cells. The DCFH-DA working concentration can be 0.1-20 μM for different cells and treatments. A pre-experiment is suggested to determine the appropriate concentration. The total dilution ratio should be more than 1:500 to avoid effects of DMSO on cells. DMSO should be set as solution control.

② Incubate at 37 °C for 30 min to a few hours, generally 30-60 min. The incubation time is related to cell types, stimulation conditions, and DCFH-DA concentration.

③ Cell collection:

Suspension cells: centrifuge the sample at 1000 xg for 5-10 min and wash with serum-free medium 2-3 times. Centrifuge and collect the cell pellet for fluorescence detection.

Adherent cells: digest the cells with 0.25% trypsin, add medium containing fetal bovine serum to terminate the digestion, thus preparing the cell suspension. Centrifuge at 1000 xg for 5-10 min and collect cells, then wash with serum-free medium 1-2 times. Centrifuge and collect cell pellet for fluorescence detection.

2. Fluorescence detection:

① Re-suspend collected cells with serum-free medium for detection.

② Wavelength: the excitation wavelength is 488 nm, the emission wavelength is 525 nm. It can also be detected according to the fluorescence detection conditions of FITC.

**Note:** The density of re-suspended cells 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.

Detection of tissue sample

1. Preparation of single cell suspension:

Method 1: using the single cell suspension instrument.

Method 2: enzyme digestion.

① Take the tissue into pre-cooled PBS (0.01 M, pH 7.4) immediately and clean the blood and other contaminants. Remove the massive composition, fiber, fat, and blood vessels (except for specialized cells).

② Cut the tissue into approximately 1 $mm^3$ pieces with ophthalmic scissors, then place these pieces in pre-cooled PBS (0.01 M, pH 7.4) to remove cell debris.

③ Add an appropriate amount of enzyme for digestion, incubate in 37 °C water bath for 20-30 min and gently oscillate the mixture intermittently.

④ Stop the digestion with medium containing fetal bovine serum. Filter the mixture to remove the tissue massive components with nylon mesh (300 mesh) and collect the cells. Centrifuge at 500 xg for 10 min and discard the supernatant, then wash with PBS (0.01 M, pH 7.4) 1-2 times. Re-suspend to prepare the single cell suspension solution. The cell count should be no less than $10^6$.

Method 3: mechanical method.

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

② Secure the nylon mesh (300 mesh) on a small beaker, then place the tissue pieces on the mesh and gently rub the tissue with ophthalmic scissors or erasing knife. Wash the tissue with PBS (0.01 M, pH 7.4) at the same time.

③ Collect the cell suspension and centrifuge at 500 xg for 10 min. Then discard the supernatant and wash with PBS (0.01 M, pH 7.4) 1-2 times. Re-suspend to prepare the single cell suspension solution. The cell count should be no less than $10^6$.

2. Add the fluorescent probe:

① Add DCFH-DA working solution to the cells. The DCFH-DA working concentration can be 0.1-20 μM for different cells and treatments. A pre-experiment is suggested to

determine the appropriate concentration. The total dilution ratio should be more than 1:500 to avoid effects of DMSO on cells. DMSO should be set as solution control.

② Incubate at 37 °C for 30 min to a few hours, generally 30-60 min. The incubation time is related to cell types, stimulation conditions, and DCFH-DA concentration.

③ Collect the incubated single cell suspension, centrifuge at 1000 xg for 5-10 min to collect cells. Wash with serum-free medium 1-2 times. Centrifuge and collect the cell pellet for fluorescence detection.

3. Fluorescence detection:

① Re-suspend collected cells with serum-free medium for detection.

② Wavelength: the excitation wavelength is 488 nm, the emission wavelength is 525 nm. It can also be detected according to the fluorescence detection conditions of FITC.

# 8. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

**Assay Genie 100% money-back guarantee!**

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

**Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.**