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ab238535
DCF ROS/RNS Assay Kit (biofluids, culture supernatant, cell lysates)

For a sensitive method to detect total reactive oxygen species (ROS) plus reactive nitrogen species (RNS) in a wide variety of sample types, such as serum, plasma, urine, cell lysates or cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

DCF ROS/RNS Assay Kit (biofluids, culture supernatant, cell lysates) (ab238535) is an assay for measuring the total free radical presence of a sample.

The assay employs a proprietary quenched fluorogenic probe, dichlorodihydrofluorescin DiOxyQ (DCFH-DiOxyQ), which is a specific ROS/RNS probe that is based on similar chemistry to the popular 2', 7'-dichlorodihydrofluorescein diacetate. The DCFH-DiOxyQ probe is first primed with a quench removal reagent, and subsequently stabilized in the highly reactive DCFH form. In this reactive state, ROS and RNS species can react with DCFH, which is rapidly oxidized to the highly fluorescent 2', 7'-dichlorodihydrofluorescein (DCF). Fluorescence intensity is proportional to the total ROS/RNS levels within the sample. The DCFH-DiOxyQ probe can react with hydrogen peroxide (H_2O_2), peroxyl radical ($ROO^{\cdot}$), nitric oxide (NO), and peroxynitrite anion (ONOO-).

The kit has a detection sensitivity limit of 10 pM for DCF and 40 nM for H_2O_2 respectively. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

2. Protocol Summary

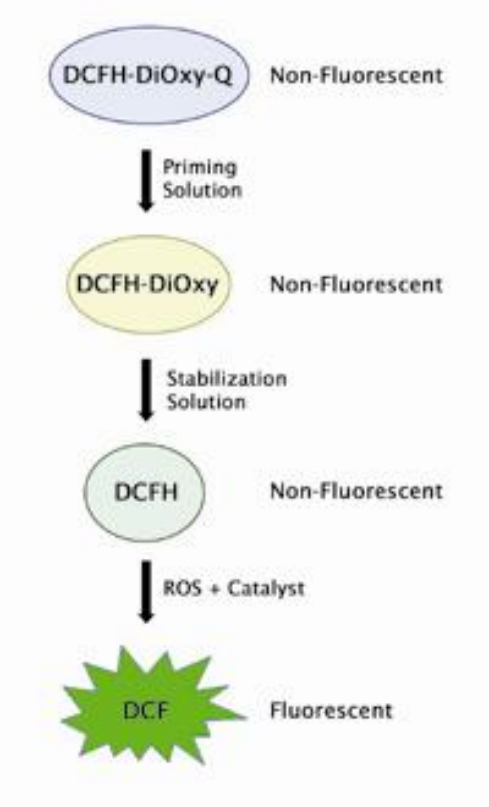

Figure 1. Mechanism of In Vitro ROS/RNS Assay.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at +4°C immediately upon receipt and check below for storage for individual components.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity (96 test)	Storage condition
Priming Reagent	250 µL	+4°C
Stabilization Solution (10X)	1.5 mL	+4°C
Catalyst (250X)	20 µL	+4°C
DCF-DiOxyQ	50 µL	-20°C
DCF Standard	100 µL	-20°C
Hydrogen Peroxide	100 µL	+4°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Phosphate Buffered Saline for sample preparations and dilutions
- 96-well black or fluorescence microtiter plate
- Fluorescent microplate reader capable of reading 480 nm (excitation) and 530 nm (emission)

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 1X Stabilization Solution:

6.1.1 Dilute the 10X Stabilization Solution 1/10 by adding 1.5 mL of solution to 13.5 mL of deionized water. Stir or vortex to homogeneity. Store the solution at 4°C.

6.2 1X Catalyst:

6.2.1 Prior to use, dilute the 250X Catalyst 1/250 in PBS. Vortex thoroughly. Prepare only enough for immediate applications (eg. add 10 µL of Catalyst to 2.49 mL PBS for 50 wells).

6.3 DCFH Solution

6.3.1 Prepare only enough DCFH Solution for immediate applications in an amber tube or aluminum foil covered tube.

6.3.2 Prepare DCFH Solution by diluting the stock solution of DCF-DiOxyQ 1/5 with Priming Reagent (eg. for 50 assays, add 25 µL DCF-DiOxyQ to 100 µL Priming Reagent). Vortex to homogeneity. Incubate the solution for 30 mins at room temperature.

6.3.3 Next, dilute the reaction 1/40 with 1X Stabilization Solution (eg. for 50 assays, add 125 µL DCF-DiOxyQ/Priming Reagent reaction to 4.875 mL of Stabilization Solution).

6.3.4 Vortex to homogeneity. Protect the solution from light. This solution is now stable in the DCFH form and ready to use. The solution may be stored at -20°C for up to one week when protected from light.

Δ **Note:** Due to light-induced auto-oxidation, the stock DCF-DiOxyQ solution and all subsequent DCF-DiOxy and DCFH solutions must be protected from light.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Preparation of the DCF Standard Curve

7.1.1 Prepare a 1/10 dilution series of DCF standards in the concentration range of 0 µM – 10 µM by diluting the 1mM DCF stock in 1X PBS.

7.1.2 Transfer 200 µL of each DCF standard to a 96-well plate suitable for fluorescence measurement.

7.1.3 Read the relative fluorescence with a fluorescence plate reader at 480 nm excitation / 530 nm.

Standard #	DCF Standard (µL)	PBS (µL)	DCF (nM)
1	10	990	10,000
2	100 of standard #1	900	1,000
3	100 of standard #2	900	100
4	100 of standard #3	900	10
5	100 of standard #4	900	1
6	0	1000	0

Δ **Note:** The DCF standard curve is optional and is used to ensure that the plate reader is working properly and that the dye can be detected at various concentrations; however the DCF standards do not go through the assay protocol and cannot give any indication of free radicals in a sample. The hydrogen peroxide standard curve is more useful because it is used to measure the hydrogen peroxide levels in the sample, which is the predominant form of ROS and will be the majority of the signal detected in the sample.

It is fine to use the DCF standard curve if you are interested in presenting your results as relative comparisons between samples. The DCF standard curve does not give any indication of free radicals in a sample and a DCF value is not meaningful, so it is probably not a good idea to present your result values as DCF (nM).

If you have to present your results as absolute values, you should use the hydrogen peroxide standard curve, which will reflect the amount of hydrogen peroxide in the samples.

7.2 Preparation of the H_2O_2 Standard Curve

7.2.1 To prepare the Hydrogen Peroxide standards, first perform a 1/4,400 dilution of the stock Hydrogen Peroxide in deionized water. Use only enough for immediate applications (eg. Add 5 µL of Hydrogen Peroxide to 22 mL deionized water). This solution has a concentration of 2 mM.

7.2.2 Use the 2 mM H_2O_2 solution to prepare standards in the concentration range of 0 µM – 20 µM by further diluting in PBS.

7.2.3 H_2O_2 diluted solutions and standards should be prepared fresh. Use the table below as a reference guide only. The volumes and concentrations of the standard may be adjusted by the user.

Standard #	2 mM H_2O_2 Standard (µL)	PBS (µL)	H_2O_2 (µM)
1	10	990	20
2	500 of standard #1	500	10
3	500 of standard #2	500	5
4	500 of standard #3	500	2.5
5	500 of standard #4	500	1.25
6	500 of standard #5	500	0.625
7	500 of standard #6	500	0.313
8	500 of standard #7	500	0.156
9	500 of standard #8	500	0.078
10	500 of standard #9	500	0.039
11	0	1000	0

8. Sample Preparation

General sample information:

- The assay may be used on cell or tissue lysates, cell culture supernatants, serum, plasma, urine, and other biological fluids.
- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range. Use PBS for dilution and preparation of samples.
- We recommend that you use fresh samples for the most reproducible assay, due to the transient nature of ROS.
- If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C for up to 1-2 months.
- Some common detergents and denaturants have been tested for compatibility in the assay (below table). Dilution of samples, and interfering substances, may be necessary for assay compatibility.

Substance	Compatible Concentration
Triton X-100	<1%
NP-40	<1%
SDS	<0.1%
Deoxycholate	<1%
Tween-20	<0.1%
EDTA	<10 mM
EGTA	<10 mM
Glycerol	<10%

8.1 Cells or Tissues:

8.1.1 Resuspend cells at 1-2 x 10^7 cells/mL or tissues at 10-50 mg/mL in PBS.

8.1.2 Homogenize or sonicate on ice. To remove insoluble particles, spin at 10,000 g for 5 min. The homogenate can be assayed directly or stored at -80°C as necessary.

8.2 Serum, Plasma, Urine or Cell Culture Supernatants:

8.2.1 To remove insoluble particles, spin at 10,000 g for 5 min. The supernatant can be assayed directly or stored at -80°C as necessary.

9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

9.1 Add 50 µL of unknown sample or hydrogen peroxide standard to wells of a 96-well plate suitable for fluorescence measurement.

9.2 Add 50 µL of Catalyst to each well. Mix well and incubate 5 minutes at room temperature.

9.3 Add 100 µL of DCFH solution to each well. Cover the plate reaction wells to protect them from light and incubate at room temperature for 15-45 minutes.

9.4 Read the fluorescence with a fluorescence plate reader at 480 nm excitation / 530 nm emission.

10. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

10.1 Average the duplicate reading for each standard, control and sample.

10.2 Subtract the mean value of the H_2O_2 blank (Standard #10) from all standards, controls and sample readings. This is the corrected absorbance.

10.3 If significant, subtract the sample background control from sample readings.

10.4 Plot the corrected values for each standard as a function of the final concentration of H_2O_2.

10.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can

plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

10.6 Apply the corrected sample OD reading to the standard curve to get H_2O_2 concentration in the sample wells.

10.7 Normalized concentration of H_2O_2 in [B units / W units] in the test samples is calculated as:

$$\text{Normalized } H_2O_2 \text{ concentration} = \frac{B}{W} * D$$

Final unit: µM H_2O_2 / µg protein

Where:

B = concentration of H_2O_2 in the sample well calculated from standard curve in µM

W = protein amount in the sample well in µg

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

11. Typical Data

The following figures demonstrate typical Free Radical ROS/RNS Assay results. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

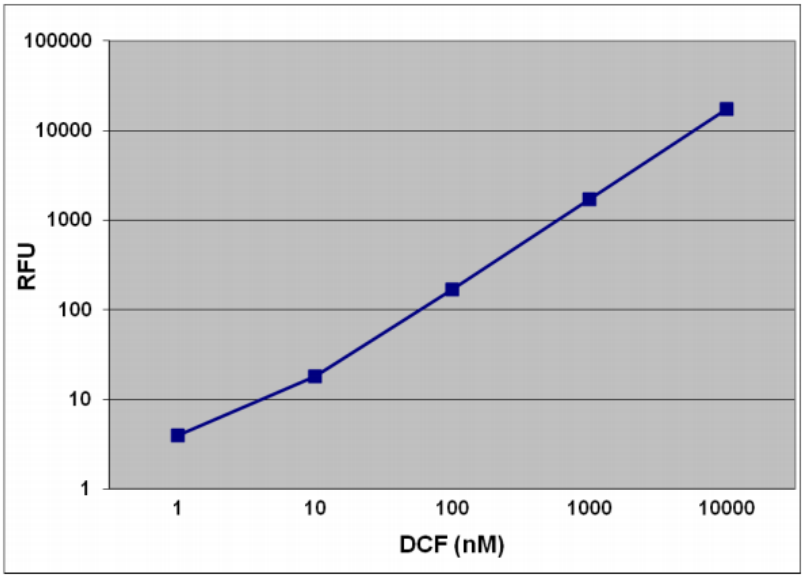

Figure 2. DCF Standard Curve.

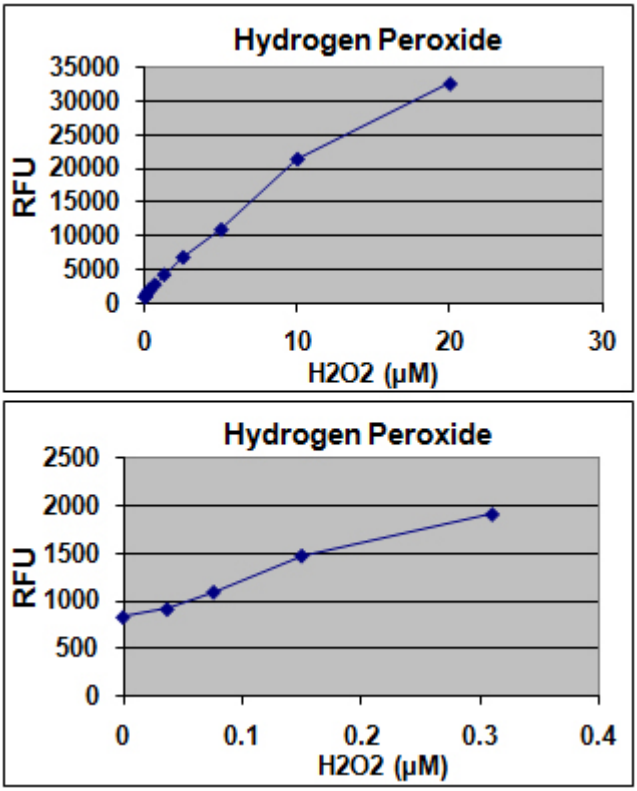

Figure 3. Hydrogen Peroxide Standard Curve.

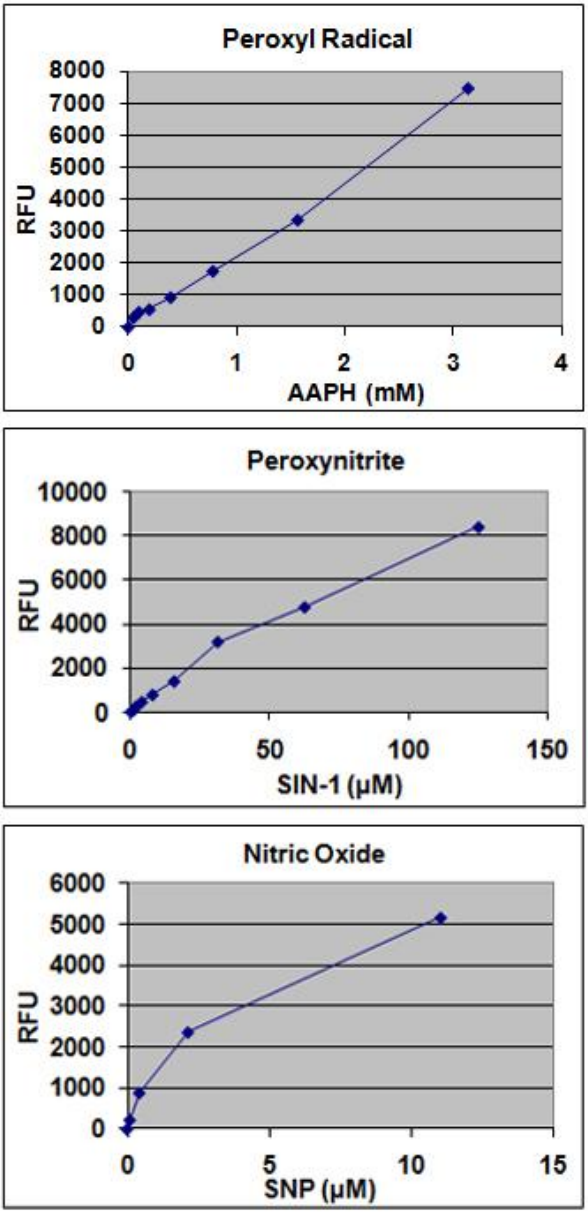

Figure 4. Detection of Various Free Radical Species. DCF fluorescence curves for AAPH (peroxyl radical generator), SIN-1 (peroxynitrite generator), and SNP (nitric oxide generator).

12. Notes

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)