

Apr 01, 2025

Intracellular ROS Assay

DOI

<https://dx.doi.org/10.17504/protocols.io.4r3l268e3v1y/v1>

Karyna Tarasova¹, Sinan Gültekin², iris.gerner Gerner², Florian Jenner²

¹Veterinary Medicine University; ²Vetmeduni Vienna



Karyna Tarasova

Veterinary Medicine University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.4r3l268e3v1y/v1>

Protocol Citation: Karyna Tarasova, Sinan Gültekin, iris.gerner Gerner, Florian Jenner 2025. Intracellular ROS Assay. protocols.io <https://dx.doi.org/10.17504/protocols.io.4r3l268e3v1y/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: April 01, 2025



Last Modified: April 01, 2025

Protocol Integer ID: 125867

Keywords: Senescence, ROS, oxidative stress, intracellular ros assay accumulation of reactive oxygen species, role of oxidative stress, oxidative damage to biomolecule, increase in oxidative stress, oxidative damage, intracellular ros assay accumulation, reactive oxygen species, damaged nucleic acid, replacement of damaged nucleic acid, senescence, other reactive species, pathogenesis, free radical, pathogenesis of several disease state, biomolecule

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to [protocols.io](https://www.protocols.io) is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with [protocols.io](https://www.protocols.io), can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

Accumulation of reactive oxygen species (ROS) coupled with an increase in oxidative stress has been implicated in the pathogenesis of several disease states. The role of oxidative stress in senescence is well established. Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to biomolecules, a process held in check by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids.

Guidelines

Preparation of Reagents

- 1X DCFH-DA: Dilute the 20X DCFH-DA stock solution to 1X in cell culture media, preferably without FBS. Stir or vortex to homogeneity. Prepare only enough for immediate applications.

Notes:

- 1X DCFH-DA/media solution contains 5% methanol. For cells that are sensitive to methanol, we recommend instead preparing a 0.1X (100 μ M) solution of DCFH-DA in cell culture media.
- Due to light-induced auto-oxidation, DCFH-DA solutions at any concentration must be protected from light



Materials

Kit Components:

1. 20X DCFH-DA (Part No. 234201): One 500 μ L amber tube of a 20 mM solution in methanol.
2. DCF Standard (Part No. 234202): One 100 μ L amber tube of a 1 mM solution in DMSO.
3. Hydrogen Peroxide (Part No. 234102): One 100 μ L amber tube of an 8.821 M solution.
4. 2X Cell Lysis Buffer (Part No. 234203): One 20 mL bottle.

Materials Not Supplied:

1. Sterile DPBS for washes and buffer dilutions
2. Hank's Balanced Salt Solution (HBSS)
3. Cell culture medium (ie: DMEM +/-10% FBS)
4. 96-well black or fluorescence microtiter plate
5. Fluorescent microplate reader capable of reading 480 nm (excitation) and 530 nm (emission)

Before start

OxiSelectTM Intracellular ROS Assay Kit (STA-342 , Green Fluorescence) was acquired from Cell Biolabs, INC.



Standard curve preparation

1

- 1.1 Prepare a 1:10 dilution series of 2', 7'- Dichlorodihydrofluorescein (DCF) standards in the concentration range of 0 μ M – 10 μ M by diluting the 1 mM DCF stock in cell culture media according to instructions.



Assay protocol

- 2 Prepare and mix all reagents thoroughly before use according to instructions. Each unknown sample should be assayed in duplicate or triplicate.
- 2.1 Remove the media from all wells and discard. Wash cells gently with Hank's Balanced Salt Solution (HBSS) 2-3 times. Remove the last wash and discard.
- 2.2 Add 100 μ L of 1X cell-permeable fluorogenic probe 2', 7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) solution to cells. Incubate at 37°C for 60 min.
- 2.3 Remove solution. Wash with HBSS. Remove the last wash and discard.
- 2.4 Add 100 μ L of medium without fetal calf serum (FCS) to each well. Add 100 μ L of the 2X Cell Lysis Buffer, mix thoroughly and incubate 5 min.
- 2.5 Transfer 150 μ L of the mixture to a 96-well plate suitable for fluorescence measurement.
- 2.6 Read the fluorescence with a fluorometric plate reader at 480 nm/530 nm.

