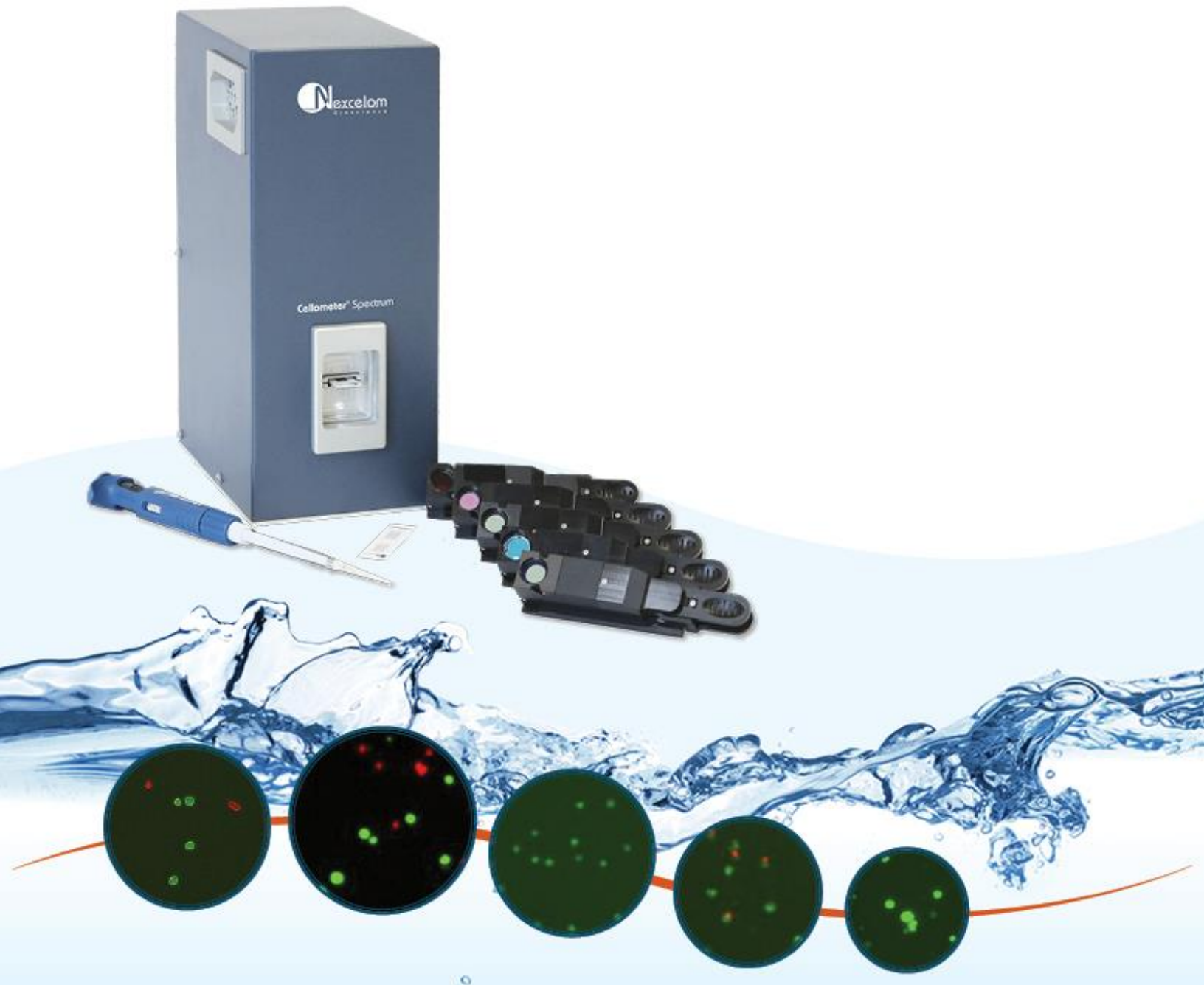


Cellometer[®] Spectrum

ViaStain[™] Total ROS Green for Cellometer Spectrum

Product Number: CSK-V0022-1



This product is for RESEARCH USE ONLY and is not approved for diagnostic or therapeutic use



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Description

Reactive oxygen species (ROS) are natural byproducts of the normal metabolism of oxygen and play important roles in cell signaling. However, during oxidative stress-related states, ROS levels can increase dramatically. The accumulation of ROS results in significant damage to cell structures. The role of oxidative stress in cardiovascular disease, diabetes, osteoporosis, stroke, inflammatory diseases, a number of neurodegenerative diseases and cancer has been well established. The ROS measurement will help to determine how oxidative stress modulates varied intracellular pathways. The ROS Assay Kit uses our unique ROS sensor to quantify ROS in live cells. ROS Green is cell-permeable. It generates the green fluorescence when it reacts with ROS. The kit is an optimized "mix and read" assay format, without a washing step.

Materials

Materials Supplied

1. CSK-V0022-1
 - a. Component A: 1 vial of lyophilized ROS
 - b. Component B: 20 mL of ROS Buffer
 - c. Component C: 200 μ L of DMSO

Materials Required

1. Micro centrifuge tube
2. Pipette
3. Cellometer SD100 or PD100 slides
4. Cellometer Spectrum

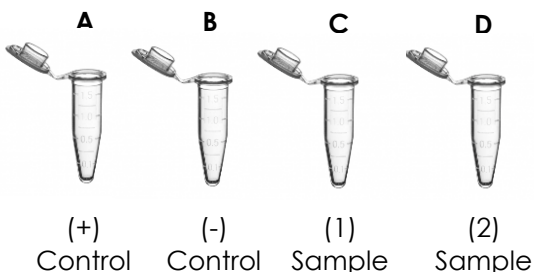
1. Prepare ROS Green Stock Solution:

Note: All unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. To ViaStain™ Total ROS Green for Cellometer Spectrum (Component A) add 110 μL of DMSO (Component C) to produce stock solution
2. Mix well, protect from light and store at -20 °C

2. Multi-Sample Experimental Protocol:

1. Add 2 μL of Total ROS Green stock solution (reconstituted component A) into 362 μL of ROS buffer (component B). This is your ROS working solution
2. Obtain 4 small empty centrifuge tubes
3. Obtain 400 μL of cells at 3×10^6 cells/ml
4. Aliquot 100 μL of cells per each empty tube
5. Centrifuge all four tubes for 5 minutes at 1200 rpm.
6. Decant media from each tube
7. Resuspend cells in 90 μL in ROS working solution from step 1.
8. Incubate for 1 hr at 37°C



9. After 1 hr incubation treat cells with desired compound(s) or move to step 11
 - a. For negative control, add equal volume of 1x PBS
 - b. For positive control, add 3.2 μL of 2.9 mM TBHP. See Section 4 for detail
10. Incubate/treat cells for an appropriate amount of time
 - a. Treat for 30 minutes at 37°C if using TBHP
11. After incubation, add 20 μL of cell sample into a Cellometer counting chamber
12. Insert into Cellometer Spectrum and image

3. Single-Sample Experimental Protocol:

1. Obtain 400 μL of cells at 3×10^6 cells/ml
2. Centrifuge cells for 5 minutes at 1200 rpm.
3. Decant media and resuspend cells in 362 μL of ROS buffer (component B)
4. Add 2 μL of Total ROS Green stock solution (reconstituted component A)
5. Incubate for 1 hr at 37°C
6. After 1 hr incubation either treat cells with desired compound or load 20 μL of cell sample into a Cellometer counting chamber
7. Insert into Cellometer Spectrum and image

4. Generation of Single-Sample Positive Control:

1. Prepare *tert*-Butyl hydroperoxide solution (TBHP 70X) working solution by adding 2 μL TBHP stock solution to 5 mL of Water (this makes a 2.9 mM working solution)
2. Obtain 400 μL of cells at 3×10^6 cells/ml
3. Centrifuge cells for 5 minutes at 1200 rpm.
4. Decant media and resuspend cells in 362 μL of ROS buffer (component B)
5. Add 2 μL of Total ROS Green stock solution (reconstituted component A)
6. Incubate for 1 hr at 37°C
7. After incubation add 13 μL of 2.9 mM TBHP to induce ROS.
 - a. Final TBHP concentration is 100 μM
 - b. **Note:** Need to add 3.2 μL of 2.9 mM TBHP to 90 μL cells (tube A, step 9 for multi-sample protocol)
8. Incubate for 30 minutes at 37°C
9. After 30 minutes load the 20 μL of treated sample into a Cellometer counting chamber
10. Insert into Cellometer Spectrum and image

Storage and Handling

Store the ROS Staining Solution at -16 to -24°C protected from light. AVOID REPEATED FREEZE THAW CYCLES. Please consult the Safety Data Sheet for more safety information, found on www.nexcelom.com/Products.

Warranty

This product is for RESEARCH USE ONLY and is not approved for diagnostic or therapeutic use. Product is warranted to meet the specifications outlined in the Certificate of Analysis when stored and used according to the manufacturer's instructions. No other warranty, expressed or implied (such as merchantability, fitness for a particular purpose, or non-infringement) is granted. Warranty is valid until the expiration date stated on the product label. If no expiration is listed, the warranty is valid for 6 months from the date of product receipt.

Warranty will be void if product is stored incorrectly, the recommended protocol is not followed, or the product is used for a different application.

Ordering Information

When ordering with a Purchase Order:

Fax a copy of the order to 978-327-5341

Email a copy of the order to sales@nexcelom.com

When ordering with a Credit Card:

Visit www.shop.nexcelom.com and place your order