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Striatal oxidative stress quantification (OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence))

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We use this protocol and it's working

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Abstract

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Attachments

STA-347-in-vitro-ros...

321KB

Oxidative stress quantification(OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence))

1 H_2O_2 is quantified using OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence) from CellBio Labs (Cat # STA-347-5) according to the manufacturer's intructions.

Sample preparation

2 Following dissection, whole striatal tissue is homogenized in 100 µL PBS using a tissue homogenizer.

3 Samples are diluted (1:25) in PBS. Different dilutions should be tested for different tissues and experimental conditions. Prepare enough for 110 microliters of diluted samples (50 ul x duplicates +spare volume).

Standards Preparation (please refer to manufacturer protocol attached)

4 Due to light-induced auto-oxidation, the stock DCFH solutions must be protected from light.

5 To prepare the Hydrogen Peroxide standards, first perform a 1:4400 dilution of the stock Hydrogen Peroxide in deionized water. Use only enough for immediate applications (e.g., Add 5 µL of Hydrogen Peroxide to 22 mL deionized water). This solution has a concentration of 2 mM.

6 Use the 2 mM H2O2 solution to prepare standards in the concentration range of 0 µM – 20 µM by further diluting in PBS (see Table 3 in attached manual). H2O2 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.

Prepare other reagents (please refer to manufacturer protocol attached)

7 1X Stabilization Solution: 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 4ºC.

8 1X Catalyst: Prior to use, dilute the 250X Catalyst 1:250 in PBS. Vortex thoroughly. Prepare only enough for immediate applications (e.g., add 10 µL of Catalyst to 2.49 mL PBS for 50 wells).

9 DCFH Solution: Prepare only enough DCFH Solution for immediate applications in an amber tube or aluminum foil covered tube. Prepare DCFH Solution by diluting the stock solution of DCF-DiOxyQ 1:5 with Priming Reagent (e.g., for 50 assays, add 25 µL DCF-DiOxyQ to 100 µL

Priming Reagent). Vortex to homogeneity. Incubate the solution for 30 minutes at room temperature. Next, dilute the reaction 1:40 with 1X Stabilization Solution (e.g., for 50 assays, add 125 µL DCF-DiOxyQ/ Priming Reagent reaction to 4.875 mL of Stabilization Solution). 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 -20ºC for up to one week when protected from light.

Assay (please refer to manufacturer protocol attached)

10 Prepare and mix all reagents thoroughly before use. Each sample, including unknown(s) and standard(s), should be assayed in duplicate or triplicate.

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

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

13 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.

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