Nile Red *UltraPure Grade*

Ordering Information	*Storage Conditions*
Product Number: 22190 (25 mg)	Keep at -20 °C and desiccated Expiration date is 12 months from the date of receipt

Introduction

Nile red (also known as Nile blue oxazone) is a lipophilic stain. It has environment-sensitive fluorescence. Nile red is intensely fluorescent in a lipid-rich environment while it has minimal fluorescence in aqueous media. It is an excellent vital stain for the detection of intracellular lipid droplets with fluorescence microscopy and flow cytometry. Nile red stains intracellular lipid droplets red. Better selectivity for cytoplasmic lipid droplets can be obtained when the cells are viewed with yellow-gold fluorescence (450-500 nm excitation; >528 nm emission) rather than red fluorescence (515-560 nm excitation; >590 nm emission). Nile red is strongly fluorescent, but only in a hydrophobic environment.

Chemical and Physical Properties

Molecular Weight: 318.37
Solvent: Dimethylsulfoxide (DMSO)
Spectral Properties: Ex/Em = 552/636 nm

Assay Protocol with Nile Red

Brief Summary

Prepare cells with test compounds → Add 200 to 1000 nM Nile Red working solution → Incubate at room temperature or 37 °C for 5 to 10 min → Remove the Nile Red working solution → Read fluorescence intensity at Ex/Em = 552/636 nm

Note: Following is our recommended protocol for live cells. This protocol only provides a guideline, and should be modified according to your specific needs.

1. Prepare Nile Red working solution:

1.1 Prepare a 1 mM stock solution of Nile Red in high-quality, anhydrous DMSO. The stock solution should be used promptly; any remaining solution should be aliquoted and frozen at < -20 °C.
Note: Avoid repeated freeze-thaw cycles, and protect from light.

1.2 Prepare a 200 to 1000 nM working solution of 1X Nile Red right before use by diluting Nile Red DMSO stock solution (from Step 1.1) in Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice, pH 7. Mix them well by votexing.

2. Run Nile Red with a fluorescence microscope or a flow cytometer:

2.1 Treat cells with test compounds for a desired period of time.

2.2 Centrifuge the cells to get 1-5 × 10^5 cells per tube.

2.3 Resuspend cells in 500 µL of Nile Red working solution (from Step 1.2).

2.4 Incubate at room temperature or 37 °C for 5 to 10 min, protected from light.

2.5 To remove the Nile Red working solution from the cells, wash the cells with HHBS or buffer of your choice. Resuspend cells in 500 µL of prewarmed HHBS or medium to get 1-5 × 10^5 cells per tube.

2.6 Monitor the fluorescence change at Ex/Em = 552/636 nm with a fluorescence microscope or a flow cytometer.

Note 1: For adherent cells, cells can be washed with HHBS or buffer of your choice, and loaded with Nile Red working solution directly in the cell plate.
Note 2: Cells can also be prefixed, and then stained with Nile Red working solution.

***Disclaimer:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact our technical service representative for more information.*