

Version 1 Last updated 15 March 2018

ab228553 Nile Red Staining Kit

For the measurement of intracellular lipid droplets using fluorescence microscopy, flow cytometry or a fluorescence microplate reader.

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

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1. Overview

ab228553 Nile Red Staining Kit is designed for the quantitative, fluorometric detection and measurement of intracellular lipid droplets using fluorescence microscopy, flow cytometry or a fluorescence microplate reader.

Adherent cells

Prepare the Nile Red Staining Solution as directed and equilibrate to room temperature.



Aspirate culture media and add an equal volume of Nile Red Staining Solution. Incubate at 37°C/5% CO₂ for 10-30 minutes.



Remove Nile Red Staining Solution.



Read fluorescence at Ex/Em = 550/640 nm with a microplate reader or observe the cells using a fluorescence microscope with a TRITC filter set.

Suspension cells

Prepare the Nile Red Staining Solution as directed and equilibrate to room temperature.



Pellet cells ($1 - 5 \times 10^5$) by centrifugation at 1000 rpm for 5 minutes.
Resuspend cells in 500 μ L Nile Red Staining Solution.



Incubate at room temperature or 37°C for 10-30 minutes in the dark.



Pellet cells by centrifugation and remove Nile Red Staining Solution.
Resuspend cells in 500 μ L pre-warmed buffer/culture medium.



Monitor the fluorescence increase using fluorescence microscope with a TRITC filter set or flow cytometer at FL1 channel.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaw of reagents.

| Item | Quantity | Storage temperature (before prep) |
|-----------------|----------|-----------------------------------|
| Nile Red | 40 µL | -20°C |
| Staining Buffer | 20 mL | -20°C |

Δ Note: 20 µL Nile Red is sufficient for 1 x 96 well plate.

3. Materials Required, Not Supplied

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

- Low speed centrifuge.
- CO₂ incubator.
- Fluorescence microscope with TRITC filter set.
- Flow cytometer.
- Fluorescence microplate reader.

4. 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.

5. Reagent Preparation

Briefly centrifuge Nile Red vial at low speed prior to opening.

5.1 Nile Red Staining Solution

1. Warm Nile Red to room temperature.
2. Warm Staining Buffer to room temperature.
3. Add 2 μ L Nile Red to 1 mL Staining Buffer to generate the working Nile Red Staining Solution.

5.2 Staining Buffer

1. Ready to use as supplied.

6. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

6.1 Staining adherent cells

1. Grow cells either in a 96-well black wall/clear bottom plate (100 μ L/well) or on cover-slips inside a petri dish filled with the appropriate culture medium.
2. Gently aspirate the culture medium, and add equal volume (such as 100 μ L/well) of the Nile Red Staining Solution.
3. Incubate the cells in a 37°C in a 5% CO₂ incubator for 10-30 minutes.
4. Remove Nile Red Staining Solution by aspiration.
Δ Note: Since Nile Red has minimal fluorescence in aqueous media, aspiration of the growth medium (Step 2) and removal of Nile Red staining solution (Step 4) after staining is optional.
5. Read Fluorescence at 550/640 nm with a microplate reader or observe the cells using a fluorescence microscope with a TRITC filter set.

6.2 Staining suspension cells

1. Centrifuge the cells at 1000 rpm for 5 minutes to get $1-5 \times 10^5$ cells per tube.
2. Resuspend cells in 500 μL of Nile Red Staining Solution (Section 5).
3. Incubate at room temperature or 37°C for 10 to 30 minutes, protected from light.
4. Centrifuge to remove the Nile Red Staining Solution, and resuspend cells in 500 μL of pre-warmed medium or buffer of your choice to give $1-5 \times 10^5$ cells per tube.

Δ Note: Since Nile Red has minimal fluorescence in aqueous media, removal of Nile Red staining solution (Step 4) after staining is optional.

5. Monitor the fluorescence increase using a fluorescence microscope with a TRITC filter set or a flow cytometer at FL1 Channel.

Δ Note: Stained cells can be fixed with 3-4% formaldehyde. In addition, prefixed cells (3-4% formaldehyde fixation) can be stained with Nile Red Staining Solution.

7. Typical Data

Data provided for demonstration purposes only.

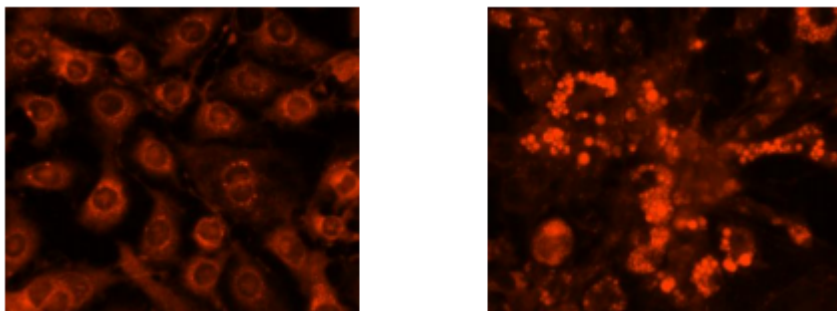


Figure 1. Fluorescence images of intracellular lipid droplets in 3T3-L1 fibroblasts (left panel) and 3T3-L1 adipocyte cells (right panel) using ab228553 Nile Red Staining Kit. The fluorescence signal was measured using a fluorescence microscope with a TRITC filter.

8. Notes

Technical Support

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