ab287838 – Lipid Staining Kit

For the Staining of neutral lipids in cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287838

Storage and Stability

All components in this kit are shipped on blue ice and are suitable for storage at room temperature, unless reconstituted. Upon receipt, immediately store kit at room temperature in the dark. Individual components may be stored at alternative temperatures as shown in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Wash Buffer II	50 mL	Ambient or -20°C
10% Formalin	24 mL	Ambient
Oil Red O	1 vial	Ambient
Hematoxylin	24 mL	Ambient

Materials Required, Not Supplied

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

- Syringe and 0.2 µm syringe filter or Whatman No. 1 filter paper
- Light microscope
- 100% isopropanol
- 96-well clear plate with flat bottom (optional)
- Multi-well spectrophotometer (ELISA reader) (optional)

Reagent Preparation

Wash Buffer II, 10% Formalin, and Hematoxylin: Store at -20°C and ready to use as supplied. Stable for 1 year.

Oil Red O: To make Oil Red O Stock Solution, dissolve Oil Red O in 20 ml of 100% isopropanol, mix well and let it sit for 20 min. Stable for 1 year. To make Oil Red O Working Solution, add 3 parts of Oil Red O Stock Solution to 2 parts of dH_2O, mix well, and allow to sit for 10 min. Filter with 0.2 µm syringe filter or Whatman No. 1 paper or equivalent. Prepare the working solution 15 min before use. Working solution is stable for 2 hrs.

Assay Protocol

- All components: For 96-well plate, use 100 µl per well. For 24-well plate, use 500 µl per well. For 6-well plate, use 2 ml per well & for 100 mm culture dish, use 6 ml/dish.

Cell Fixing:

1. Remove media from cells and gently wash 2X with Wash Buffer II. Add 10% Formalin to each well and incubate for 30 min to 1 hr.

Δ Note: *Do not pipet directly onto cells, pipet to the side of well or plate and mix by rotating.*

Cell Staining:

1. Prepare Oil Red O Working Solution as mentioned above.
2. Remove 10% Formalin and gently wash cells 2X with dH_2O for 5 min.
3. Make Isopropanol (60%) by adding 3 parts Isopropanol (100%) to 2 parts of water. Add Isopropanol (60%) to each well and incubate for 5 min
4. Remove isopropanol and add Oil Red O Working Solution to cover the cells completely and evenly.
5. Rotate plate or dish and incubate for 10-20 min.
6. Remove Oil Red O solution and wash 2-5X with dH_2O as needed until excess stain is no longer apparent.
7. Add Hematoxylin and incubate for 1 min.
8. Remove Hematoxylin and wash with dH_2O 2-5X as needed.
9. Keep cells always covered with dH_2O and while viewing under microscope.
10. Lipid droplets appear red, and nuclei appear blue.

Δ Note: *Discard used Oil Red O Solution. Re-use of Oil Red O Solution results in poor staining quality.*

Quantification (Optional):

1. Oil Red O staining can be measured semi-quantitatively. After staining with Hematoxylin and washing with dH_2O, wash additional 3X with 60% isopropanol.
2. Wash each time for 5 min, with gentle rocking.
3. Extract Oil Red O stain with 100% isopropanol for 5 min, with gentle rocking.
4. It is recommended to use at least 24-well plate, as smaller wells will give lower signal.
5. Extract with 50% normal volume (e.g., for 24-well plate, use 250 µl per well) and use 80% of extraction volume to measure.
6. Use 100% isopropanol as background control to subtract the background signal. Read absorbance at 492 nm.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)