Technical Bulletin

Live Cell Fatty Acid Staining Kit

Catalogue number MAK597

Product Description

Lipid droplets (LDs) are dynamic, ubiquitously present lipid-storage organelles, predominantly present in adipocytes. Triglycerides, neutral lipids, and cholesterol esters stored in LDs are the largest sources of energy. The presence of excess LDs in adipocytes is linked to different stress conditions and pathologies of cells and tissues (such as: Obesity, dyslipidemia, diabetes type 2, diabetic nephropathy, hepatic toxicity, and cancer).

The Live Cell Fatty Acid Staining Kit is suitable for selective staining and detection of neutral lipids in cultured cells and tissues. The Nucleic Acid Staining Solution (DAPI) included in the kit stains the nuclei of the cells whereas Fatty Acid Staining Solution stains the lipid droplets.

Components

The kit is sufficient for 4 x 6-well or 16 x 24-well cell culture plates.

- Washing Buffer 10X 25 mL
  Catalogue Number MAK597A
- Nucleic Acid Staining Solution 25 mL
  Catalogue Number MAK597B
- Fatty Acid Staining Solution 500 µL
  Catalogue Number MAK597C

Reagents and Equipment Required but Not Provided

Live cells

For cell growth:

- 6-well or 24-well culture plates, sterile with lid
- Pipettors and Pipettes
- Vortex Mixer
- Gloves

For assay:

- Aluminum foil to protect from light.
- Fluorescent microscope e.g. Olympus IX71 or equivalent.

Slides

- Isopropanol (Catalogue number I9516 or equivalent)
- Filter paper Whatman No .1 (Catalogue number WHA1001125 or equivalent).
- Histology slides- mouse liver/rat liver
- Pipettors, multi-Pipettors, dispensers, appropriate tips, troughs, microtubes
- Deparaffinization solution.
- Formalin fixed paraffin-embedded tissue sections (5-7 µm), prepared for immunostaining (SOP 10-139R)
- Glycerol-Gelatin (Catalogue number GG1 or an equivalent aqueous mounting medium) preheated at 37 °C/56 °C
- 'Wax' Pen (such as PAP Pen Catalogue number Z377821, Zymed 00-8888, DAKO Pen S 2002)
- Slide holder
- Coverslips No.1 (24x32 mm or 22x22 mm, such as Sigma C9802)
- Timer
- Fluorescent microscope such as Olympus IX71 or equivalent.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product is shipped on dry ice. Store at -20 °C upon receipt protected from light.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. Avoid repeated freeze/thaw cycles.

Washing Buffer 10X (MAK597A)

Allow buffer to come to room temperature and before use dilute the required amount 10-fold with ultrapure water.

Nucleic Acid Staining Solution (MAK597B)

Bring the solution to room temperature before use. After use, store at -20 °C. Ensure that the solution is protected from light.

Fatty Acid Staining Solution (MAK597C)

Allow to thaw and bring the solution to room temperature before use.

For live cell staining, dilute the required aliquot 100-fold with 1X washing buffer.

For fixed slides, dilute the required aliquot 10-fold with 1X washing buffer. After use store the undiluted solution at -20 °C. Ensure that the solution is protected from light.

Procedure

It is essential to protect both the Nucleic Acid Staining Solution and the Fatty Acid Staining Solution from light by preparing the solutions in a light protected vessel.

Sample Types

These reagents may be used on live cells and fixed histology slides.

Protocol for Staining Live Cells

Prepare a suitable plate for cell culture by pre-coating the wells with 0.1% gelatin or collagen solutions.

1. Add the desired medium to each well (3 ml for 6-well plates and 1 ml for 24-well plates)
2. Sow the desired number of cells to each well (between 10^4 to 10^5 cells per well)
3. Grow the cells for at least 24 hours.
4. Induce the fatty acid bodies in the cells (using either IBMX/Dexamethasone[1] (Catalogue number IBMX I7018); Dexamethasone D4902; Insulin I0516) or oleic acid[2,3,4] (O1008).
5. After the required length of induction remove the medium and wash with Washing Buffer 1X (3 x 1 ml/well for 6-well plates and 3 x 0.25 ml/well for 24-well plates)
6. Prepare the Fatty Acid staining solution at 100-fold dilution and add to each well (1 ml/well for 6-well plates or 0.25 ml/well for 24-well plates). Protect the plate from light by covering it with aluminum foil, and incubate for 30 minutes at 37°C.
7. Wash the plates three times as before with 1X Washing buffer.
8. Add the Nucleic Acid staining solution to each well (1 ml/well for 6-well plates or 0.25 ml/well for 24-well plates). Protect the plate from light by covering it with aluminum foil and incubate for 5 minutes at 37 °C.
9. Wash the plates three times as before with 1X Washing buffer. Leave 1 ml or 0.25 ml of the washing buffer in each well.
10. Observe the cells in a fluorescent microscope.

Record the results in this order:

Brightfield

Fatty Acid Stain: λ_{ex} 493 nm/λ_{em} 519 nm

DAPI: λ_{ex} 358nm/ λ_{em} 461nm

The nucleic acid stain will show blue fluorescence, and the fatty acid stain will show green fluorescence.

Protocol for Staining of FFPE Histology Slides

Take FFPE (formalin-fixed, paraffin embedded) slides of either rat liver or mouse liver sections, and perform deparaffinization in the chemical hood, per the manufacturer's instructions.

1. Use a 'Wax' Pen to circle the tissue sections.
2. Wash slides with the diluted 1X Washing Buffer for 3 times for 5 minutes.
3. Stain with the Fatty Acid Staining solution, (250 µl per tissue section, 5 µg/ml in PBS) and incubate for 30 minutes at RT protected from light.
4. Rinse the Fatty Acid Staining Solution off using purified water and then wash 3 times for 5 minutes using diluted Washing Buffer.
5. Stain with the Nucleic Acid Staining Solution (MAK597B) using 250 µl for 5 mins at 37 °C protected from light.
6. Wash slides 3 times with diluted Washing Buffer and allow to dry at room temperature prior to fluorescence microscopy analysis.

Fatty Acid Stain: λ_{ex} 493 nm/λ_{em} 519 nm

DAPI: λ_{ex} 358nm/λ_{em} 461 nm

Let the coverslip dry for 10-20 minutes before analysis.'

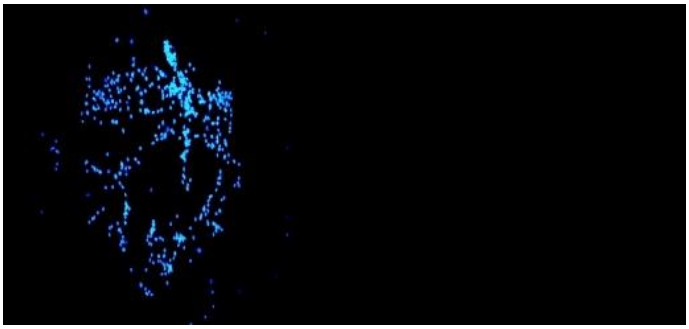
Use a fluorescent microscope and take images with the desired magnification.

The nucleic acid stain will show blue fluorescence, and the fatty acid stain will show green fluorescence.

Results

Figure 1.
Induction of fatty acid bodies in 3T3-L1 cells with IBMX/Dexamethasone. Undifferentiated Cells of 3T3-L1 (cells in pre adipocyte state) were cultured and differentiated using IBMX-DEX (1 µM DEX, 0.5 mM IBMX) method to gain adipocyte phenotype. Cells were stained with the Fatty Acid Staining Solution (MAK597C) and Nucleic Acid Staining Solution (MAK597B) as seen using fluorescent microscopy.

Uninduced 3T3-L1 cells



Induced 3T3-L1 cells

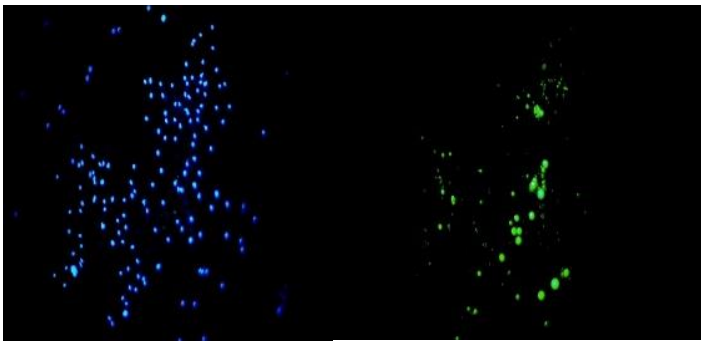
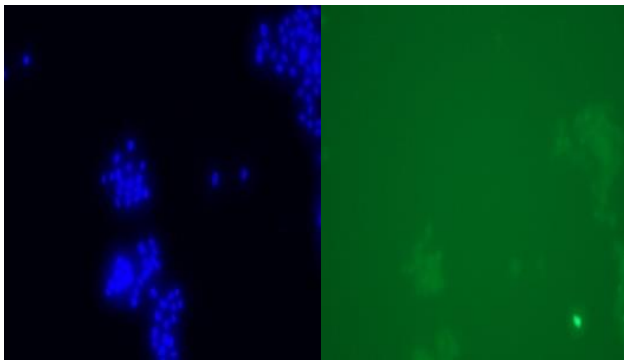


Figure 2.

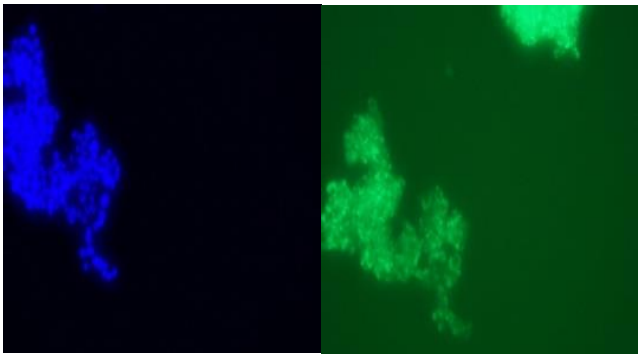
Induction of fatty acid bodies in HepG2 cells with Oleic acid.

Undifferentiated Cells of HepG2 were cultured and differentiated using 0.2% Oleic acid. Cells were stained with Fatty Acid Staining solution (MAK597C, blue) and Nucleic Acid Staining Solution (MAK597B, green) as seen using fluorescent microscopy.

Uninduced HepG2 cells



Induced HepG2 cells



All samples and standards should be run in technical duplicates or triplicates.

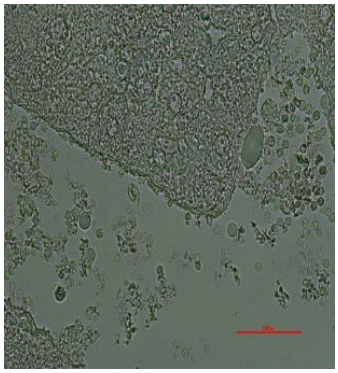
Figure 3.

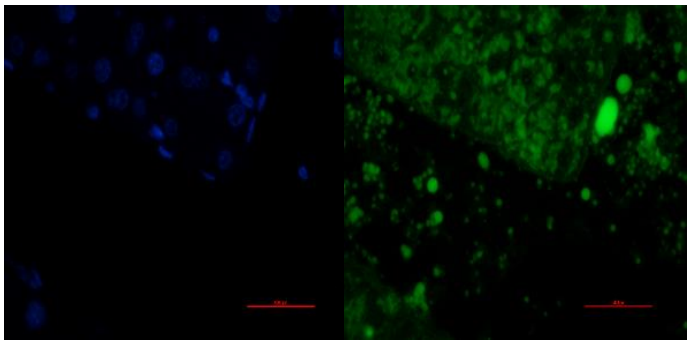
Staining a slide of rat liver.

Histology slides of rat liver X400 magnification 33ms exposure, Brightfield.

Blue- Nucleic acid staining solution

Green- Fatty Acid staining solution





Notes:

1) The Fatty Acid Stain may give a background of green autofluorescence.
2) It is essential to protect the plates from light (after staining) during all stages until observing in the fluorescent microscope.
3) Since the cells are live, the position of the stained cells observed with both stains may be **shifted slightly and not coincident** between views.

References

1) Reed BC and Lane MD, Insulin receptor synthesis and turnover in differentiating 3T3-L1 preadipocytes. *Proc. Natl. Acad. Sci.,* **77,** 285-289, (1980)
2) Listenberger LL.,*et al.*, Fluorescent detection of lipid droplets and associated proteins*, Curr. Protocols Cell Biol.,* **71** 4.31.1-4.31.14. (2016).
3) Eynaudi A.,*et al.*, Differential effects of oleic acid and palmitic acids on lipid droplet-mitochondria interaction in the hepatic cell line HepG2, *Frontiers in nutrition*, **8**, Article 775382, (2021).
4) Moravcova A.,*et al.*, The effect of oleic and palmitic acid on induction of steatosis and cytotoxicity on rat hepatocytes in primary culture, *Physiol. Res*. **64** (Suppl. 5), S627-S636. (2015).

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mak597pis Rev 05/25