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Inspiration for Life Science

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Catalog No. PMC-AK19F-COS

Adipocyte Fluorescent Staining kit

Introduction

The Adipocyte Fluorescent Staining Kit (Cat.No.AK19F) is designed to stain both lipid droplets and nuclei in adipocytes using BODIPY® (Invitrogen Corporation) and H33258, respectively. Furthermore, the amount of lipid per cell and cell shape can be quantified using imaging instrument such as IN Cell Analyzer 1000 (GE Healthcare Company)

Components

Component	Quantity	Storage
Tablet for washing	5 tablets	4-10ºC
BODIPY®	50mL	4-10ºC
H33258	50mL	4-10ºC
mounting agent	50mL	4-10ºC

One kit contains regents for 10 × 96-well plates

Additional Materials Required

・fixative solution

37 % formaldehyde	100 ml
Distilled or deionized water	900 ml
NaH2PO4(H2O)	4 g
Na2HPO4	6.5 g

Preparation of washing buffer

Add one **Tablet for washing** to 100 ml of distilled or deionized water and mix.
Note: This working solution can be stored at 4°C for 7 days.

Protocol (96-well plate format)

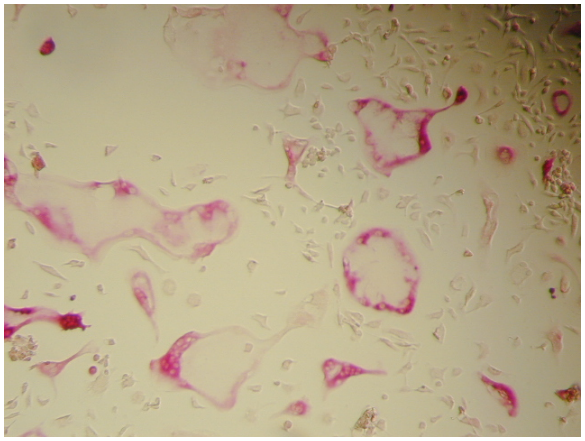
1. Remove culture medium. Wash each well once with 100uL of washing buffer.
2. Add 50uL of fixative solution to each well and fix overnight at room temperature.
3. Remove fixative solution. Wash each well with 100uL of washing buffer. (× 2 times)
4. Add 50uL of BODIPY to each well and incubate for 30 minutes at room temperature.
5. Remove BODIPY. Add 50uL of H33258 to each well and incubate for 30 minutes at room temperature.
6. Remove H33258. Wash each well once with 100uL of washing buffer.
7. Add 50uL of mounting agent to each well.
8. Examine the sample by fluorescence microscope.

Note: For detection of lipid droplets, examine cells with an excitation filter of 498 nm and an emission filter of 503 nm.

For detection of nucleus, examine cells with an excitation filter of 352 nm and an emission filter of 461 nm.

Application examples

Visceral preadipocytes collected from a Spraguee Dawley rats (male) were differentiated into mature adipocytes and subjected to staining of lipid droplets (Figure 1) and nuclei (Figure 2).



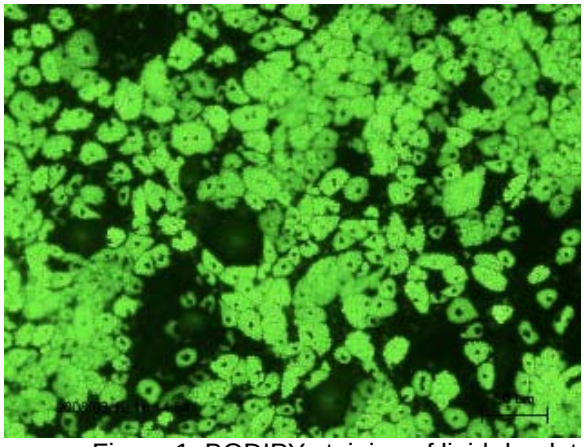


Figure 1: BODIPY staining of lipid droplets

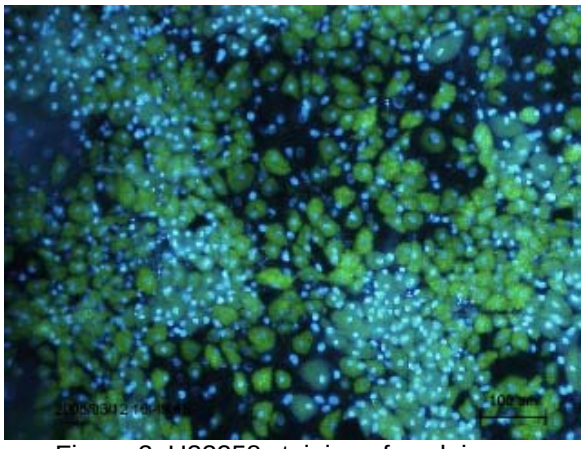


Figure 2: H33258 staining of nuclei

References

(1) Sunao Takesita, Keisuke Kaji, Akira Kudo. Identification and Characterization of the New Osteoclast Progenitor with Macrophage Phenotypes Being Able to Differentiate into Mature Osteoclasts. JOURNAL OF BONE AND MINERAL RESEARCH Volume 15, Number 8 (2000). 1477-1488.
(2) BEN A. A. SCHEVEN, JONE S. MILNE, SIMON P. ROBINS. A SEQUENTIAL CULTURE APPROACH TO STUDY OSTEOCLAST DIFFERENTIATION FROM NONADHERENT PORCINE BONE MARROW CELLS. In Vitro Cell. Dev. Biol. July-August (1998). Animal 34: 568-577.

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