CELLBIO™
in service of your CELL

Technical Datasheet

EZStain™ Adipocyte Staining Kit

Product Code: CCK013-1KT

1. Introduction

Adipose tissue in humans is composed of loose connective tissue and adipocytes. Changes in adipocyte size and/or number result in changes in the mass of adipose tissue within the body. Adipogenesis is the process of development of adipocytes from pre-adipocytes or re-engineered fibroblasts or mesenchymal stem cells and is achieved through proliferation and differentiation of pre-adipocytes. Adipocytes or adipogenesis can be visualized or quantified by the ready-to-use staining reagents supplied in the kit.

2. About the Kit

Oil Red O or Sudan IV belongs to a family of lipophilic or fatty acid soluble dyes. These dyes are used to demonstrate triglycerides, lipids and lipoproteins. Oil-Red-O is generally used to detect presence of fat globules i.e. identification of adipocytes within tissue or adipocytic differentiation of cells.

3. Applications

- Identification of adipocytes in tissue
- Identification of novel cellular factors / pathways involved in adipogenic differentiation
- Evaluation of effects of trophic factors, cytokines, and growth promoters, hormones, hormonal analogs and steroids on the differentiation process

4. Kit contents

Code	Contents	Quantity
CCK013(A)	Washing Solution	1 X 20ml
CCK013(B)	Fixing Solution	1 X 20ml
CCK013(C)	Permeabilization Solution	1 X 20ml
CCK013(D)	Oil-Red-O staining solution	1 X 20ml

Store all the reagents at room temperature

5. Materials required but not provided in the kit

- Adipocytes / Adipose tissue
- Sterile water
- Microscope with 40X or higher objectives.
- Microscopy slides
- Cover slips
- Scalpel
- Multi-well plates
- Serological pipettes
- Spectrophotometer or a 96-well plate reader capable of measuring the absorbance at 500nm

6. Directions for use

Users are advised to review entire procedure before starting the assay

Suggested working volumes of all reagents according to culture vessel

Culture vessel	Volume per well	No. of tests performed using 1 kit
96-well plate	75µl	260
48-well plate	150µl	130
24-well plate	300µl	60
12 well plate	500µl	40
6 well plate	1ml	20

6.1 General guidelines

- Do not leave the cell monolayer dry for more than 30 seconds during entire staining procedure.
- Gently add and remove all the reagents from the culture vessel to avoid detachment of cells from vessel surface. Add the reagents along the side of culture wells.
- Oil-Red-O stains skin and clothing. Wear PPE (Personal Protective Equipment) while handling the solution.
- Controls:
  Include appropriate controls-
  - Negative control: Undifferentiated cells
  - Positive control: Cells differentiated with known differentiation reagent

6.2 Procedure for staining cultured cells in plates

6.2.1 Washing the cells

1. Aspirate off the spent medium from control wells and adipogenic differentiated wells.
2. Add appropriate volume of washing solution along the side of each well.
3. Swirl gently to wash the cell layer.

6.2.2 Fixation and permeabilization of cells

1. Aspirate off the washing solution and add appropriate volume of fixing solution to each well.
2. Incubate the plate at room temperature for 30 - 60 minutes in fume hood.
3. After incubation aspirate off the fixing solution and add appropriate volume of distilled water along the side of each well.
4. Swirl gently to remove any traces of fixing solution.
5. Aspirate off the water and add appropriate volume of permeabilization solution.
6. Incubate the plate at room temperature for 5 minutes.

6.2.3 Staining the cells

Preparation of working stain solution

1. Mix 3 parts of Oil-Red-O staining solution with 2 parts of distilled water.
2. Incubate for 10 minutes at room temperature.
3. Filter the working Oil-Red-O solution through Whatmann filter paper
   Note: Working stain solution is stable for only 2 hours. Prepare only the required quantity just prior to use.

Procedure for staining

1. After incubation aspirate off the permeabilization solution and add appropriate volume of staining solution.
2. Incubate the plate at room temperature for 5 minutes.
3. After incubation aspirate off the staining solution and add appropriate volume of distilled water along the side of each well.
4. Swirl gently to remove any traces of staining solution.
5. Aspirate off the water and repeat washing with water till clear solution is obtained.
6. Again add distilled water to each well and observe under phase contrast microscope at 40X magnification.

6.3 Procedure for quantification of staining

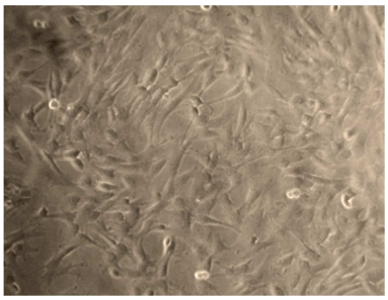
1. Aspirate off the water from wells.
2. Add 100% isopropyl alcohol.
3. Incubate at room temperature for 1 minute.
4. Measure absorbance at 500nm using isopropyl alcohol as blank on spectrophotometer or 96-well plate reader.

6.4 Procedure for staining tissue

1. Place a very thin piece of tissue on a clean, grease-free microscopy slide.
2. Mince it with the help of scalpel.
3. Very small unminced pieces of tissue can be left on the slide.
4. Place the slide on a sheet of tissue paper.
5. Put staining solution on the tissue in a quantity sufficient to cover the tissue.
6. Place long coverslip on the tissue across the slide.
   *(**Note**: Avoid trapping of bubbles while placing the coverslip in tissue. Presence of bubbles may interfere with microscopic observation.)*
7. Press the coverslip uniformly across the length of the slide to squash the tissue between coverslip and slide.
8. Incubate at room temperature for 30 minutes.
9. Fix the coverslip on slide with the help of nail polish.
10. Observe under phase contrast microscope at 40X magnification.

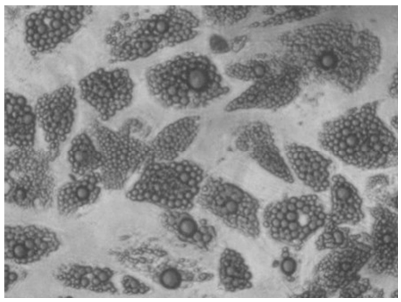
7. Interpretation of Observations

Figure 1



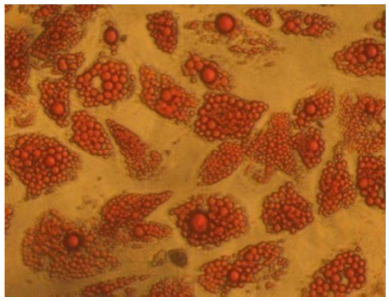
Undifferentiated Human Adult Mesenchymal Stem cells unstained with Oil Red O (40X)

Figure 2(A)



Human adult mesenchymal stem cells differentiated into adipocytes before staining with Oil Red O (40X)

Figure 2 (B)



Human adult mesenchymal stem cells differentiated into adipocytes after staining with oil red O Lipid droplets stained bright red/orange with Oil-Red-O staining solution (40X)

8. Storage and Shelf Life

- Store all the reagents at room temperature.
- If precipitation occurs in staining solution, filter it through Whatmann filter paper before use. Precipitation and subsequent filtration does not affect performance of the staining solution.
- Use before expiry date given on the label.

9. Related products:

HiDiff™ 3T3-L1 Differentiation kit
Code: CCK011-100NO

Xpert™ 3T3-L1 Differentiation Teaching Kit
Code: CCK021-25NO

10. Troubleshooting guide

Use the following troubleshooting guidelines for technical assistance

Problem	Cause	Solution
High background of staining in untreated cells	Inadequate washes after staining	Wash the cell layer with distilled water until it is no longer red/ pink in colour
	Precipitation in Oil-Red-O staining solution	Filter the staining solution through Whatmann filter paper before use
	Improper selection of the filter in spectrophotometer or 96-well plate reader	Choose appropriate filters
		Stain blank wells (containing media but no cells) to assess background staining
Non-uniform staining	Monolayer disturbed during addition or removal of media and reagents	Perform addition and removal gently along the side walls of the wells
	Cells growing in patches	Use uniformly spread confluent cells for staining

In vitro diagnostic medical device

CE Marking

Consult instructions for use

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited, B /4-6 , MIDC, Dindori, Nashik MH

www.himedialabs.com

EC REP

CE Partner 4U ,Esdoornlaa 13, 3951 DB Maarn The Netherlands,
www.cepartner 4u.eu

PIMBPCR017_O/0616 MBPCR017-02

HiMedia Laboratories Pvt. Ltd.
Plot No. C40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane (West) 400604, Maharashtra, India.Tel No.022-69034800
Email: atc@himedialabs.com Website: www.himedialabs.com.