Product Manual

CytoSelect™ 96-Well Adipogenesis Assay Kit (Colorimetric/Fluorometric)

Catalog Number

CBA-290	400 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Obesity is a risk factor in many chronic diseases including atherosclerosis, type 2 diabetes, cancer, and osteoarthritis. The ability to regulate the cell cycle and differentiation of adipocytes is important in the understanding of obesity. Adipogenesis is the process during which preadipocytes develop into mature adipocytes in a well-orchestrated, multistep process that requires the sequential activation of numerous transcription factors. The best characterized *in vitro* adipogenesis model is the 3T3-L1 cell line. These cells have been used extensively to investigate insulin-induced glucose uptake and mechanisms of obesity development. When grown under normal conditions, 3T3-L1 cells display a fibroblast-like phenotype; however, when treated with a combination of IBMX (3-isobutyl-1-methylxanthine), Insulin, and Dexamethasone, the cells undergo terminal differentiation resulting in a series of morphological and biochemical changes resulting in a rounded phenotype and formation of intracellular lipid droplets.

Cell Biolabs' CytoSelect™ 96-well Adipogenesis Assay Kit is a simple assay that quantitatively measures lipid droplet accumulation in cultured cells of the 3T3-L1 model system. Oil Red O and Nile Red Stains are included for colorimetric and fluorometric quantitation respectively. Each kit provides the reagents required for induction/detection of adipogenesis and a high-throughput method to screen activators and inhibitors. Reagents are sufficient for 400 assays in a 96-well plate format, but may be scaled to 48-well or 24-well plate formats.

Related Products

1. CBA-240: CytoSelect™ Cell Viability and Cytotoxicity Assay Kit
2. CBA-252: CytoSelect™ MTT Cell Proliferation Assay Kit
3. STA-384: Total Cholesterol Assay Kit
4. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
5. STA-618: Free Fatty Acid Assay Kit (Colorimetric)

Kit Components

Box 1 (shipped at room temperature)

1. Fixation Solution (Part No. 122402): Two 20 mL bottles
2. Oil Red O Colorimetric Stain (Part No. 129004): One 25 mL bottle
3. Extraction Solution (Part No. 129005): One 25 mL bottle

Box 2 (shipped on blue ice packs)

1. IBMX Solution, 1000X (Part No. 129001): One 500 µL sterile vial of 3-isobutyl-1-methylxanthine
2. Insulin Solution, 1000X (Part No. 129002): One 500 µL sterile vial of Human Insulin
3. Dexamethasone Solution, 10,000X (Part No. 129003): One 100 µL sterile vial
4. Nile Red Fluorometric Stain, 100X (Part No. 129006): One 700 µL amber vial

Materials Not Supplied

1. 3T3-L1 Preadipocyte Cell Line (available through ATCC)
2. DMEM containing 10% FBS
3. Adipogenesis inducers or inhibitors
4. PBS
5. 37ºC Incubator, 5% CO_2 Atmosphere
6. Light microscope
7. 96-well microtiter plate reader (490-520 nm) for Oil Red O colorimetric detection
8. 96-well tissue culture fluorescence microtiter plate
9. Fluorescent microplate reader capable of reading 485 nm (excitation) and 555 nm (emission) for Nile Red fluorometric detection

Storage

Upon receipt, store the IBMX, Insulin, Dexamethasone, and Nile Red Fluorometric Stain at -20ºC. Aliquot if necessary to avoid multiple freeze/thaw cycles. All other kit components should be stored at room temperature.

Preparation of Reagents

- Adipogenesis Differentiation Media: Add IBMX Solution, Insulin Solution, and Dexamethasone at 1:1000, 1:1000, and 1:10,000 dilutions respectively into DMEM containing 10% FBS. Store at 4ºC for up to 6 weeks.
- Insulin Media: Dilute Insulin Solution at 1:1000 in DMEM containing 10% FBS. Store at 4ºC for up to 6 weeks.
- Oil Red O Working Solution: Immediately before use, dilute 6 parts of Oil Red O Colorimetric Stain with 4 parts deionized water. Mix well. Prepare only enough for immediate applications. Do not store diluted solutions.
- Nile Red Fluorometric Stain: Immediately before use, dilute Nile Red Fluorometric Stain at 1:100 in DPBS. Mix well. Prepare only enough for immediate applications. Do not store diluted solutions.

Assay Protocol (must be under sterile conditions)

I. Colorimetric Detection with Oil Red O

The following assay protocol is written for a 96-well format. Refer to Table 1 below for the appropriate dispensing volumes of other plate formats.

Culture Dish	96-well	48-well	24-well
Preadipocyte Seeding Volume (µL/well)	100	200	400
Adipogenesis Differentiation Media (µL/well)	200	400	800
Insulin Media (µL/well)	200	400	800
PBS Wash (µL/well)	200	400	800
Fixation Solution (µL/well)	100	200	400
Oil Red O Working Solution (µL/well)	100	200	400
Extraction Solution (µL/well)	50	100	200

Table 1: Dispensing Volumes of Different Plate Formats: Oil Red O

1. Harvest and resuspend cells at 1 – 4 x 10^5 cells/mL in DMEM containing 10% FBS.
2. Seed 100 µL in each well of a 96-well plate and incubate at 37ºC, 5% CO_2 until fully confluent.
3. Gently aspirate the culture media and replace with 200 µL of Adipogenesis Differentiation Media (see Preparation of Reagents) or fresh DMEM containing 10% FBS (negative control).
4. Incubate for 3 days at 37ºC, 5% CO_2.
5. Gently aspirate the media and replace with 200 µL of Insulin Media (see Preparation of Reagents) or fresh DMEM containing 10% FBS (negative control).
6. Incubate for 2 days at 37ºC, 5% CO_2.
7. Repeat steps 5-6.
8. Observe the intracellular lipid droplet accumulation by microscopy. If additional differentiation is desired, repeat steps 5-6 until desired results are met.
9. Gently aspirate the media and wash wells once with 200 µL of PBS.
10. Add 100 µL of Fixation Solution to each well, incubating 5 minutes at room temperature.
11. Promptly remove the Fixation Solution by gently aspirating and wash wells twice with 200 µL of PBS.
12. Add 100 µL of diluted Oil Red O Working Solution (see Preparation of Reagents) to each well. Mix on an orbital plate shaker for 15-30 minutes at room temperature.
13. Wash 3 times with 200 µL of PBS.
    Note: Stained lipid droplets may be viewed under the microscope.
14. Add 50 µL of Extraction Solution to each well and mix on an orbital plate shaker for 15 minutes at room temperature.
15. Read the absorbance of each well at 490-520 nm.
    Note: For 24 and 48-well formats, transfer 50 µL to a 96-well plate for measurement.

II. Fluorometric Detection with Nile Red Stain

The following assay protocol is written for a 96-well format. Refer to Table 2 below for the appropriate dispensing volumes of other plate formats.

Culture Dish	96-well	48-well	24-well
Preadipocyte Seeding Volume (µL/well)	100	200	400
Adipogenesis Differentiation Media (µL/well)	200	400	800
Insulin Media (µL/well)	200	400	800
PBS Wash (µL/well)	200	400	800
Fixation Solution (µL/well)	100	200	400
1X Nile Red Fluorometric Stain (µL/well)	100	200	400

Table 2: Dispensing Volumes for Different Plate Formats: Nile Red

1. Harvest and resuspend cells at 1 – 4 x 10^5 cells/mL in DMEM containing 10% FBS.
2. Seed 100 µL in each well of a 96-well plate and incubate at 37ºC, 5% CO_2 until fully confluent.
3. Gently aspirate the culture media and replace with 200 µL of Adipogenesis Differentiation Media (see Preparation of Reagents) or fresh DMEM containing 10% FBS (negative control).
4. Incubate for 3 days at 37ºC, 5% CO_2.
5. Gently aspirate the media and replace with 200 µL of Insulin Media (see Preparation of Reagents) or fresh DMEM containing 10% FBS (negative control).
6. Incubate for 2 days at 37ºC, 5% CO_2.
7. Repeat steps 5-6.
8. Observe the intracellular lipid droplet accumulation by microscopy. If additional differentiation is desired, repeat steps 5-6 until desired results are met.
9. Gently aspirate the media and wash wells once with 200 µL of PBS.
10. Add 100 µL of Fixation Solution to each well, incubating 5 minutes at room temperature.
11. Promptly remove the Fixation Solution by gently aspirating and wash wells twice with 200 µL of PBS.
12. Add 100 µL of 1X Nile Red Fluorometric Stain (see Preparation of Reagents) to each well.
13. Cover the plate wells to protect the reaction from light. Mix on an orbital plate shaker for 15 minutes at room temperature.

    Note: Stained lipid droplets may be viewed under the microscope.
14. Read the fluorescence with a fluorometric plate reader at 485 nm/550 nm.

Example of Results

The following figures demonstrate typical results with the CytoSelect™ 96-well Adipogenesis Assay Kit. Absorbance measurements were performed on a Microplate Autoreader EL311 (Bio-Tek Instruments Inc.) with a 490 nm filter. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/555 nm filter set and 550 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

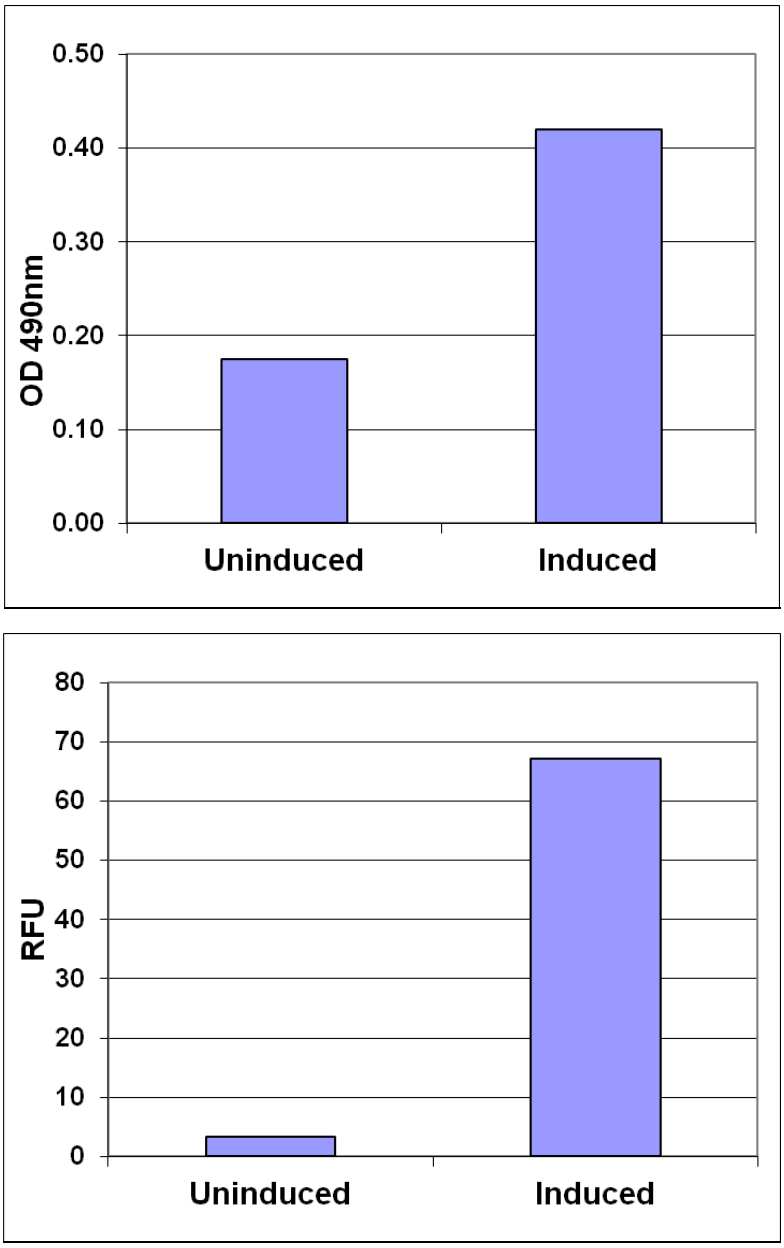

Figure 1. Differentiation of 3T3-L1 Cells. 20,000 cells/well of preadipocyte 3T3-L1 cells were seeded overnight in a 96-well plate and differentiated for 7 days. Relative adipogenesis was determined with Oil Red O colorimetric staining (top) or Nile Red fluorometric staining (bottom) as described in the Assay Protocol.

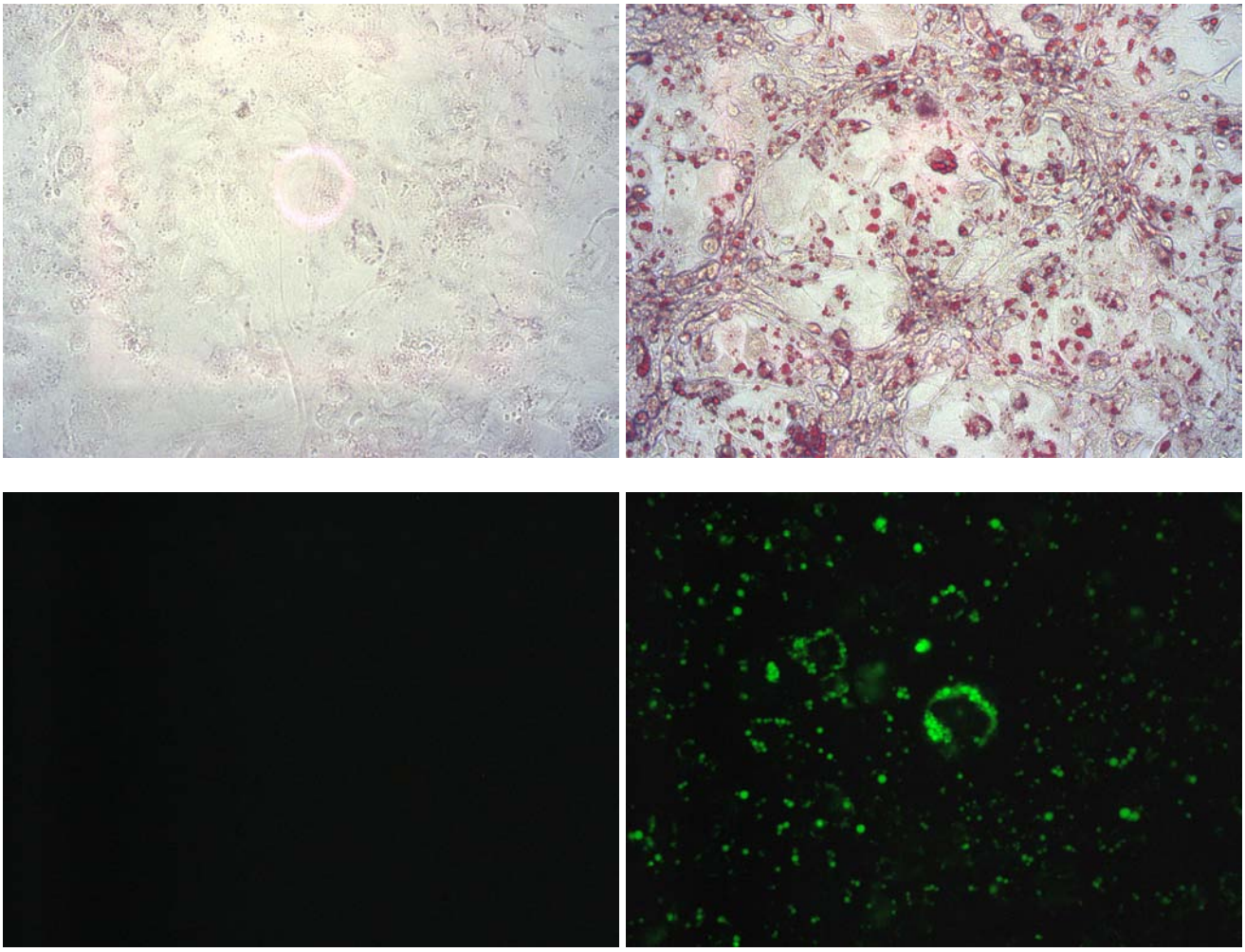

Figure 2. Differentiation of 3T3-L1 Cells. 20,000 cells/well of preadipocyte 3T3-L1 cells were seeded overnight in a 96-well plate. Wells were either uninduced (left) or induced (right) for 7 days and determined with Oil Red O staining (top) or Nile Red staining (below) as described in the Assay Protocol.

References

1. Green H. and Kehinde, O. (1975) *Cell* **5:** 19-27.
2. Rosen E.D. and Spiegelman, B.M. (2000) *Annu. Rev. of Cell and Devel. Biol.* **16**:145–171.
3. Farmer, S.R. (2006) *Cell Metab*. **4**:263–273.
4. MacDougald, O.A. and Lane, M.D. (1995) *Annu. Rev. Biochem.* **64**:345–373.

Warranty

In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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