



## Lipid Assay Kit

### Description

Oil red O stain is one of the methods for determination of differentiation in adipocyte. Oil red O, the oilphilic red dye, stains intracellular oil droplet. This lipid assay kit, consisting of cell fixative and oil red O solution, is used to stain the accumulated oil in adipocyte. Furthermore, this kit is able to quantify amount of oil with organic solvent extraction method.

### Components/Storage

Component	Quantity	Storage
Oil red O solution	150 mL×2	4°C
Solvent for Oil red O extraction	200 mL×2	4°C

One kit contains reagents for 30 × 24well plates

### Materials required but not provided

- 10% formalin or fixation

### Prepare reagents

- fixative solution

37 % formaldehyde	100 ml
Distilled or deionized water	900 ml
NaH <sub>2</sub> PO <sub>4</sub> (H <sub>2</sub> O)	4 g
Na <sub>2</sub> HPO <sub>4</sub>	6.5 g

- Oil red O working solution

1. Mix Oil red O solution and distilled water in the proportion of six to four, and leave it for 10~15 minutes at room temperature.
2. Filtrate Oil red O working solution by 0.5~1.0μm syringe filter.

**Note:** The working solution is stable for no longer than 2 hours. The solution containing crystallized Oil red O cannot be used.

### Protocols (24-well plate format)

1. Remove culture medium, and wash each well once with 500 μL of PBS.
2. Add 500μL of Fixative to each well, fix it over night at room temperature.
3. After the fixation, wash each well three times with 500μL of distilled water.
4. Add 500μL of Oil red O solution to each well, and leave at least for 15min in room temperature.
5. Remove Oil red O solution, wash each well three times with distilled water. Repeat until the distilled water is perfect clean.
6. Dry it, and observe. In addition, add 500μL of extraction reagent to each well, measure dye extraction (540 nm) by spectrophotometer or plate reader.



## **APPLICATION EXAMPLE**

**Visceral adipocytes (PMC-VAC01-COS) were stained by Lipid Assay Kit.**

Visceral adipocytes were stained with Oil red O solution (Figure 1), and then dye extractions were measured by spectrophotometer (540 nm) (Figure 2).

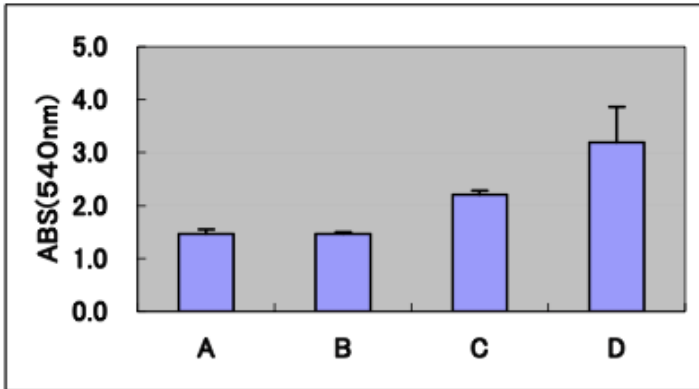


Figure 1



Figure 2

## **References**

(1) Tashiro, K., Inamura, M., Kawabata, K., Sakurai, F., Yamanishi, K., Hayakawa, T., Mizuguchi, H.

Efficient Adipocyte and Osteoblast Differentiation from Mouse Induced Pluripotent Stem Cells by Adenoviral Transduction. Stem Cells. 27, 1802-1811 (2009)

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