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Inspiration for Life Science

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Catalog No. PMC-MKV300-COS

Macrokiller V300

Introduction

Macrokiller V300 is liposomes containing clodronic acid, which has the cytocidal effect of macrophages (e.g. osteoclast and microglia). Due to the low cell permeability by itself, clodronic acid was contained into liposome to increase phagocytic efficiency of macrophages. In recent years, there are reports those macrophages play roles in many kind of diseases such as allergy, Alzheimer's disease and cancer, furthermore, in tissue regeneration. Macrokiller V300 will be a useful tool for researching these diseases by inhibiting macrophage activity.

Components

Description	Amount	Quantity	Storage Conditions	Stability
Macrokiller V300	1 mL/vial	1 vial	4°C	1 year
Empty liposomes for control				

***Shipping: 4°C**

Specifications

Concentration of clodronic acid	10 mg/mL (40.8 mmol/L)
Molecular Mass of clodronic acid	244.89
Average particle size	300 nm

Notice

1. **Avoid oxidization of liposomes (i.e. Tightly close the vial cap each time opened.)**
2. **Do NOT freeze. The freezing process destroys liposome structure.**
3. **Microscopic observation of cells will NOT be possible due to resulting cloudiness by the addition of liposomes,**

Protocol (Application Example)

A) For the primary rat microglia

1) Culture primary rat microglia as 1×10^3 cells/well in 96 well plate.
2) Incubate for 24 hours at CO_2 5%, 37°C.
3) After removing supernatant, add 0.3, 1.0, 2.0 mM clodronic acid (make Macrokiller V300 in culture medium to 1/136, 1/41, 1/20 dilutions respectively). Add empty liposomes as a control in

the same manner.

4) Incubate for 1 hour at CO_2 5%, 37°C.
5) After removing supernatant, add 200 µL culture medium heated to 37°C. Repeat supernatant removal.
6) After adding 100 µL culture medium heated to 37°C, incubate for 48 hours at CO_2 5%, 37°C.
7) Measure cell viability by the XTT assay (See example of results shown in figure 1.).

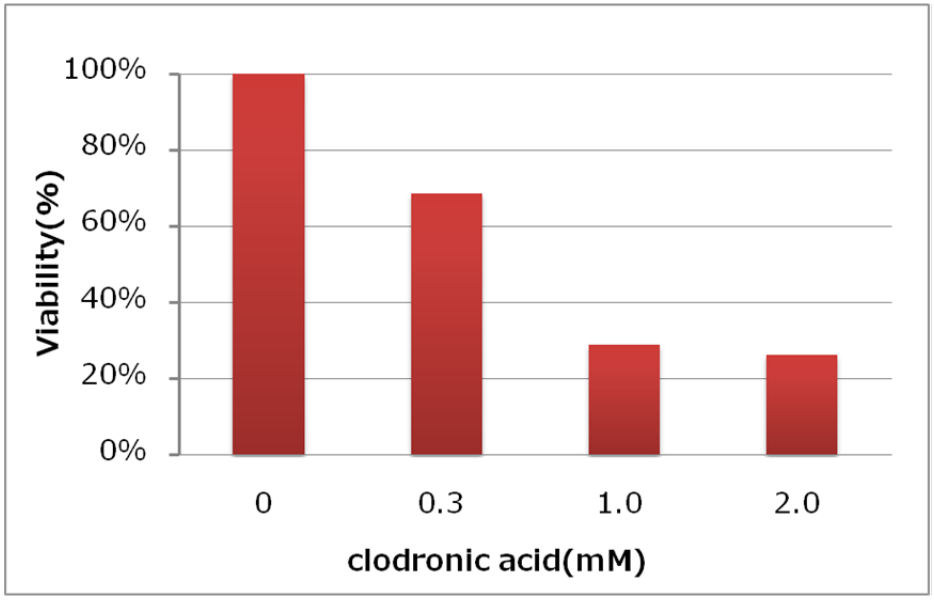

Figure 1 Cytocidal effect of Macrokiller V300 against primary rat microglia.
Dose-dependent cytocidal effect of Macrokiller V300 against primary rat microglia.
A cell viability determined by the XTT assay after 48 hours treated with Macrokiller V300.

B) For the primary rat visceral adipocyte

1) Culture visceral adipocyte as 7.5×10^4 cells/well in 48 well plate.
2) Incubate for 9 days at CO_2 5%, 37°C.
3) After removing supernatant, add 1.0 mM clodronic acid (make Macrokiller V300 in culture medium to 1/41 dilution). Add empty liposomes as a control in the same manner.
4) Incubate for 1 hour at CO_2 5%, 37°C.
5) After removing supernatant, add 500 µL culture medium heated to 37°C. Repeat supernatant removal.
6) Add 500 µL culture medium heated to 37°C and incubate for 48 hours at CO_2 5%, 37°C.
7) Add 100 ng/mL Lipopolysaccharide (LPS) into appropriate wells (See Table 1.).
8) After adding LPS, incubate for 48 hours at CO_2 5%, 37°C.
9) Remove supernatant prior to extraction of cellular TotalRNA.
10) Perform gene expression analysis (See example of results shown in figure 2.).

Table 1 Assay Example (48 well plate)

Control	-Macrokiller V300		+Macrokiller V300				
	-LPS	+LPS	-LPS	+LPS			

: Wells seeding primary rat visceral adipocyte cells

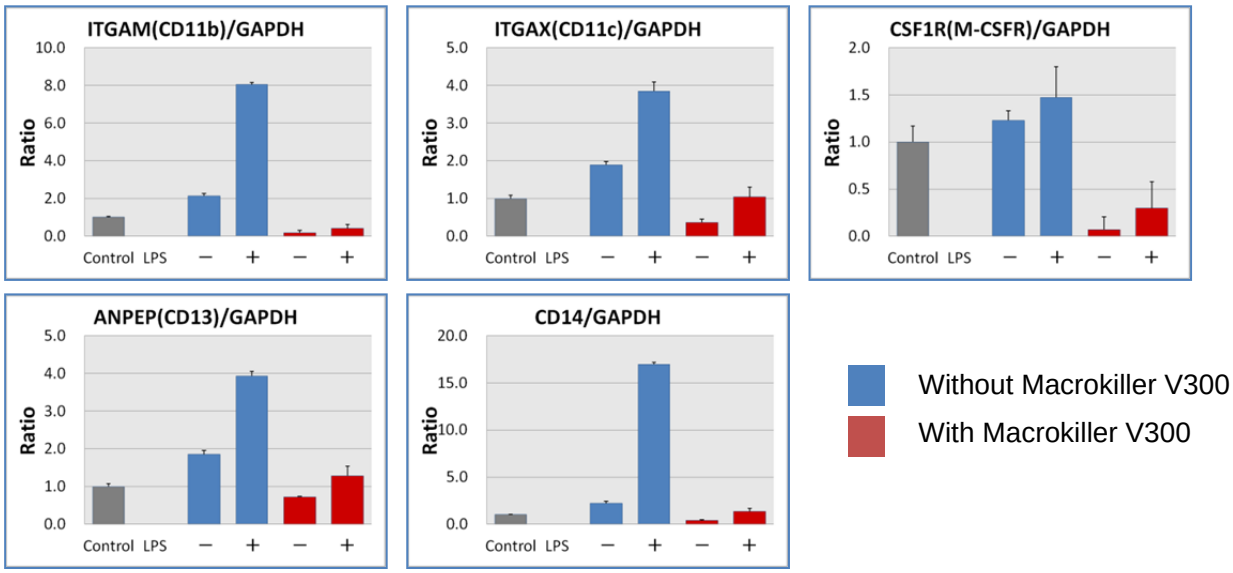

Figure 2 Selective elimination of adipose tissue macrophages from primary rat visceral adipocyte culture. Macrokiller V300 selectively decreases relative mRNA levels of macrophage marker genes

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