RAW 264.7 Polarized M1 Macrophage Induction and Identification Kit

Cat. No: XJM004 **Size: 20 Assays/100 Assays**

Kit Components

Cat.	Products	20 Assays	100 Assays	Storage
XJM004A	RAW 264.7 Cell M1 Differentiation MIX(1000×)	20 μL	100 μL	-20°C, shading light
XJM004B	RAW 264.7 Cell M1 Differentiation Detection Antibody Cocktail	100 μL	500 μL	2-8°C, shading light
XJM004C	RAW 264.7 Cell M1 Differentiation Detection Antibody Isotype Cocktail	100 μL	500 μL	2-8°C, shading light
E-AB-F0997A	Purified Anti-Mouse CD16/32 Antibody[2.4G2]	25 μg	100 μg	2-8°C, shading light
	Manual		One Copy	

Composition of Components

Products	Component
RAW 264.7 Cell M1 Differentiation Detection Antibody Cocktail	FITC Anti-Mouse F4/80 Antibody [CI:A3-1]
	PerCP/Cyanine5.5 Anti-Mouse/Human CD11b Antibody[M1/70]
	PE Anti-Mouse CD86 Antibody [GL-1]
RAW 264.7 Cell M1 Differentiation Detection Antibody Isotype Cocktail	FITC Rat IgG2b,κ Isotype Control [LTF-2]
	PerCP/Cyanine5.5 Rat IgG2b,κ Isotype Control [LTF-2]
	PE Rat IgG2a, κ Isotype Control[2A3]

Note: It is not recommended to mix Cocktail from different batches of kits.

Storage

RAW 264.7 Cell M1 Differentiation MIX(1000×) reagents can be stored for 1 year at -20°C. RAW 264.7 Cell M1 Differentiation Detection Antibody Cocktail and RAW 264.7 Cell M1 Differentiation Detection Antibody Isotype Cocktail can be stored at 2-8℃ for 1 year, Avoid freezing and repeated freeze-thaw cycles.

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Introduction

Macrophages, as heterogeneous and diverse immune cells with unique morphological and functional properties, are involved in inflammatory responses, autoimmunity, and injury repair. Macrophage polarization plays a crucial role in eliminating pathogens or maintaining tissue homeostasis. In recent years, it has been widely accepted that the development of various immune-related diseases is influenced by macrophage polarization. In a complex and variable microenvironment, macrophages can be transformed into different phenotypes. In general, macrophages have at least two different polarizations: M1-type macrophages and M2-type macrophages. Macrophages are transformed into M1-type macrophages when stimulated by substances such as lipopolysaccharide (LPS) and interferon-γ (IFN-γ). These macrophages produce high levels of nitric oxide synthase (iNOS), interleukin-6 (IL-6), and tumor necrosis factor-a (TNF-a), which promotes an inflammatory response.

RAW 264.7 (mouse monocyte macrophage leukemia cells) is one of the most commonly used in vitro models for osteoclast and inflammation studies, and is widely used to study rheumatoid arthritis, osteoporosis, osteolysis, periodontitis and other skeletal diseases.

This kit provides a complete set of differentiation and identification protocols for RAW 264.7 polarized to M1 macrophages, including M1 macrophage differentiation differentiation and identification reagents, which provides stable and reliable support for the differentiation and identification of RAW 264.7 polarized to M1 macrophages, and improves the efficiency and stability of differentiation and identification of RAW 264.7 polarized to M1 macrophages.

This kit can prepare 1 mL of RAW 264.7 differentiation medium (1 mL/well in 24-well plate) for 1 assay. 100 assays can culture about 2×10^7 RAW 264.7 cells, and about $1\text{-}2\times10^7$ M1-type macrophages with 70-90% purity can be obtained after 48h differentiation culture.

Materials Not Supplied

- **Reagents**

  DMEM (High glucose) basal medium, fetal bovine serum, penicillin-streptomycin solution, deionized water for cell culture, PBS for cell culture,0.25% Trypsin Solution.

- **Instruments**

  Centrifuge, CO_2 incubator, inverted microscope, flow cytometer, biosafety cabinet, water bath, pipettor.

- **Materials**

  Petri dish, sterile 2 mL centrifuge tubes, sterile 15mL/50mL centrifuge tubes, pipette.

Related Products

Products	Cat.	manufacturer
Cell Staining Buffer	E-CK-A107	Elabscience
DMEM (High glucose)	PM150210	Procell
Penicillin-Streptomycin Solution	PB180120	Procell
0.25% Trypsin Solution	PB180225	Procell

Other Related Products

Products	Cat.	manufacturer
Cell Stimulation and Protein Transport Inhibitor Kit	E-CK-A091	Elabscience
APC Anti-Mouse CD206/MMR Antibody[C068C2]	E-AB-F1135E	Elabscience
APC Anti-Mouse CD163 Antibody[S15049F]	E-AB-F1295E	Elabscience
APC Anti-Mouse IL-6 Antibody[MP5-20F3]	E-AB-F1207E	Elabscience

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Experimental Protocol

Note: The following operations need to be carried out under sterile conditions.

➢ Reagent preparation

1) 20% inactivated FBS- DMEM complete medium: In DMEM (High glucose) basal medium (PM150210, Procell), add penicillin solution (final concentration of 100 U/mL) and streptomycin solution (final concentration of 100 μg/mL), and add inactivated fetal bovine serum (inactivated for 1 hour at 60℃ in the water bath) at a final concentration of 20%. The prepared DMEM complete medium can be stored at 4°C for a month.
2) RAW 264.7 Differentiation Medium: Dilute RAW 264.7 Cell M1 Differentiation MIX (1000×) to 1× with 20% inactivated FBS-DMEM complete medium. For example, add 50 μL of RAW 264.7 Cell M1 Differentiation MIX (1000×) into 50 mL 20% inactivated FBS-DMEM complete medium and mix fully. The prepared RAW 264.7 differentiation medium can be stored at 4°C for 2 weeks.

➢ Differentiation culture of RAW 264.7

1) The RAW 264.7 cells were collected, centrifuged at 150g for 5 minutes, the supernatant was carefully discarded, resuspended with 2 mL RAW 264.7 differentiation medium, the cells were counted, and the cell density was adjusted to 2×10^5/mL with RAW 264.7 differentiation medium, the cells were inoculated into the cell culture dish and cultured in a 5% CO_2 incubator at 37°C for 48 h. The state of the cells was observed under the microscope daily and photographed.
2) After 48h of incubation, the cell culture supernatant in the dish was transferred to a 15 mL centrifuge tube, 0.25% trypsin solution was added to the dish to digest the cells for 1 minutes (trypsin needs to cover the cells completely, that the actual incubation time depend on the actual situation), the above retained cell culture supernatant was added to terminate the digestion, and the adherent cells were gently pipette and transferred to the 15 mL centrifuge tube, which is the M1 macrophages.

Note: After this step, you can observe whether there are cells in the petri dish under the microscope, when the cell density is around 10%, no further digestion will be carried out, otherwise it can be digested again.

3) The M1 macrophages were centrifuged at 250g for 5 minutes, the supernatant was carefully discarded, resuspended using DMEM complete medium, and the cells were counted for phenotypic characterization or conduct subsequent experiments as needed.

➢ Identification of M1 macrophages in RAW 264.7

The following operations may be performed under non-sterile conditions.

1) Label the centrifuge tubes according to the following table, add 100 μL of RAW 264.7 cells ($2\text{-}5\times10^5$ cells) to each tube, add 1 mL of Cell Staining Buffer or PBS buffer, mix fully gently, centrifuge at 250g for 5 minutes, discard the supernatant, add 100 μL of Cell Staining Buffer or PBS buffer to resuspend the cell precipitate, add 1 μL of Purified Anti-Mouse CD16/32 Antibody(1mg/mL) to each group, mix fully gently, incubate at room temperature with shading light for 15 minutes to close the FCR receptor on the cell surface of the cells, and then add the antibodies according to the following table, mix fully gently, incubate at 4°C with shading light for 30 minutes.

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Purpose of the subgroups	Sample No.	Staining grouping
Voltage adjustment	1- Blank control	Blank control (blank cells, no antibodies)
Full Panel	2-Isotype Control	5 μL RAW 264.7 Cell M1 Differentiation Detection Antibody Isotype Cocktail
	3-Test group	5 μL RAW 264.7 Cell M1 Differentiation Detection Antibody Cocktail

2) After incubation, add 1 mL of Cell Staining Buffer or PBS to each tube, mix fully gently, centrifuge at 250 g for 5 minutes, and discard the supernatant.
3) Resuspend the cells with 200 μL Cell Staining Buffer or PBS and detect with flow cytometer to and analyze.

Results Presentation

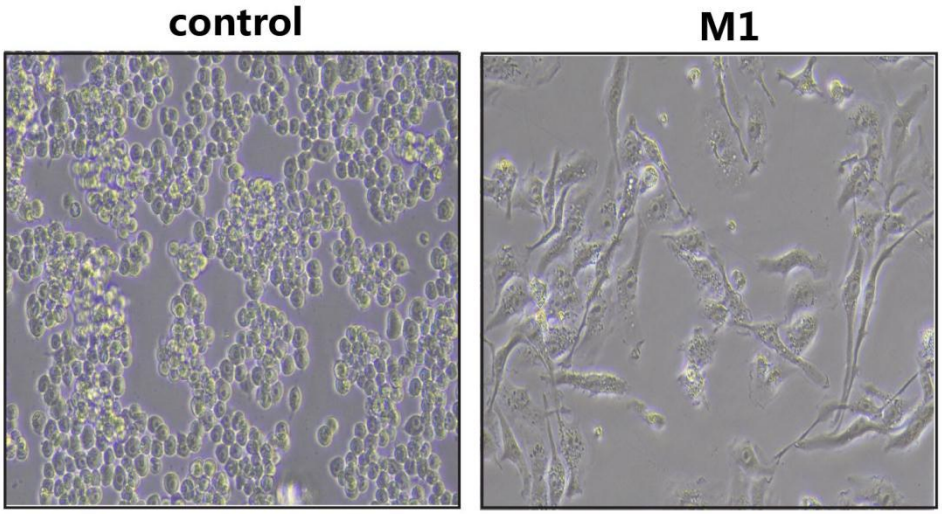

Figure 1. Morphological observations of RAW 264.7: untreated RAW 264.7 (left) cells show a round or oval morphology. RAW 264.7 Differentiated M1 macrophages (right) show irregular morphology and contain granular material in the cytoplasm.

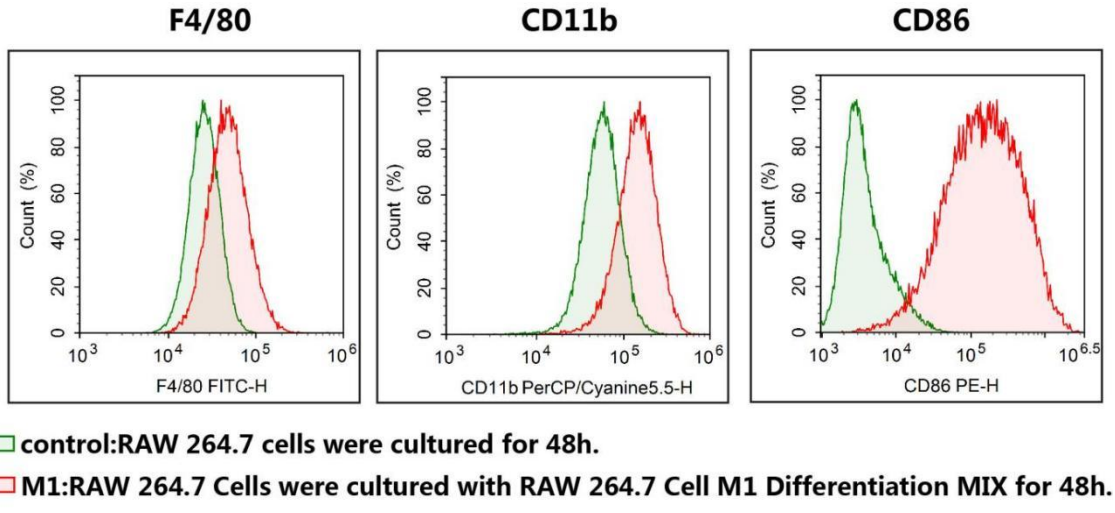

Figure 2. Phenotypic analysis of M1 macrophages of RAW 264.7: RAW 264.7 cells were induced by

polarization with RAW 264.7 Cell M1 Differentiation MIX (1000×) for 48h (M1-48h) and unpolarized treated blank RAW 264.7 cells (control-48h) were cultured for 48h with flow cytometry Detection and analysis.

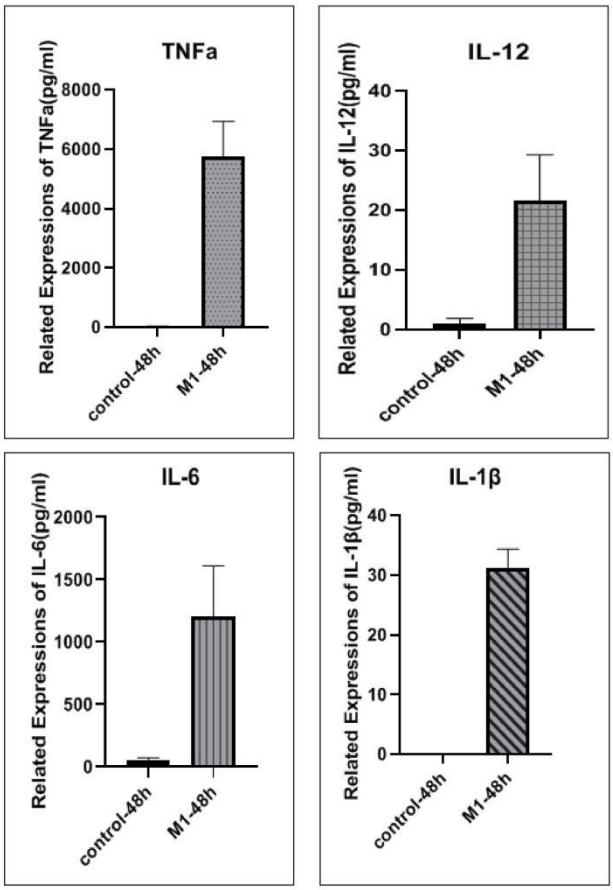

Figure 3. Cytokine expression analysis of M1 macrophages of RAW 264.7: RAW 264.7 cells were induced by polarization using RAW 264.7 Cell M1 Differentiation MIX (1000×) for 48h (M1-48h) and unpolarized blank RAW 264.7 cells (control-48h) were cultured for 48h and the supernatants were collected, and the supernatants were analyzed using the Mouse TNF-alpha (Tumor Necrosis Factor Alpha (Tumor TNF-α) ELISA Kit(E-EL-M3063)/Mouse IL-12 (Interleukin 12) ELISA Kit(E-EL-M3062)/Mouse IL-6 (Interleukin 6) ELISA Kit(E-EL-M0044) /Mouse IL-1β(Interleukin 1 Beta) ELISA Kit(E-EL-M0037) detects and analyzes the results.

Cautions

1. This product is for research use only.
2. For your safety and health, please wear laboratory overalls and disposable gloves for operation and follow the laboratory reagent operating procedures.
3. It is recommended to use the RAW 264.7 differentiation medium within 2 weeks after preparation to avoid the failure of certain components in the medium.
4. RAW 264.7 Cell M1 Differentiation MIX(1000×) can be stored at -20°C for 1 year. If not used for a long time or needs to be reused many times, it is recommended to aliquot into smaller quantities for optimal storage.
5. Excessive acceleration and deceleration of centrifuge may cause cell loss. It is suggested to adjust theacceleration no more than 3 and deceleration no more than 2, that is, Acc≦3, Dec≦2.