Mouse Bone Marrow-derived Dendritic Cells (BMDC) Induction and Identification Kit

Cat. No: XJM003 **Size: 20/200 Assays**

Cat.	Products	20 Assays	200 Assays	Storage
XJM003A	Mouse DC Cell Differentiation MIX (1000×)	20 μL	200 μL	-20℃/-80℃, shading light
XJM003B	Mouse DC Cell Maturation MIX (1000×)	10 μL	100 μL	
E-AB-F0990F	PerCP Anti-Mouse MHC II (I-A/I-E)Antibody[M5/114]]	250 μL	250 μL	2~8°C, shading light
E-AB-F0991L	Elab Fluor® 488 Anti-Mouse CD11c Antibody[N418]	250 μL	250 μL	
E-AB-F0992H	PE/Cyanine7 Anti-Mouse CD80 Antibody[16-10A1]	250 μL	250 μL	
E-AB-F0994D	PE Anti-Mouse CD86 Antibody[GL-1]	250 μL	250 μL	
E-AB-F1028E	APC Anti-Mouse CD40 Antibody[FGK4.5/FGK45]	250 μL	250 μL	
E-AB-F09842F	PerCP Rat IgG2b,κIsotype Control[LTF-2]	100 μL	100 μL	
E-AB-F09852L	Elab Fluor® 488 Armenian Hamster IgG Isotype Control[PIP]	100 μL	100 μL	
E-AB-F09852H	PE/Cyanine7 Armenian Hamster IgG Isotype Control[PIP]	100 μL	100 μL	
E-AB-F09832D	PE Rat IgG2a,κIsotype Control[2A3]	100 μL	100 μL	
E-AB-F09832E	APC Rat IgG2a,κIsotype Control[2A3]	100 μL	100 μL	
E-AB-F0997A	Purified Anti-Mouse CD16/32 Antibody[2.4G2]	50 μL	200 μL	
	Manual		One Copy	

Storage

Mouse DC Cell Differentiation MIX (1000×) and Mouse DC Cell Maturation MIX (1000×) reagents can be stored for 1 year at -20°C~-80°C; Antibodys (MHC II (I-A/I-E) and CD11c etc.)can be effectively stored at 2~8℃ for 1 year, avoiding freezing and repeated freeze-thaw.

Introduction

Mouse Bone Marrow-derived Dendritic Cells (BMDC) Differentiation and Culture Kit includes Mouse DC Cell Differentiation MIX (1000×) and Mouse DC Cell Maturation MIX (1000×),which has been developed for the in vitro culture and differentiation of Mouse Bone Marrow Cells into dendriticcells (DCs) and their subsequent maturation into fully active mature DCs.

The Mouse DC Cell Differentiation MIX (1000×) contains a combination of recombinant mouse cytokines formulated to support the differentiation of DCs from Mouse Bone Marrow Cells. It is supplied as a 1000× concentrate.

The Mouse DC Cell Maturation MIX (1000×) is formulated to support the maturation of immature DCs. It is supplied as a 1000×concentrate.

This kit enables the user to generate active mature DCs from Mouse Bone Marrow Cells in 7~8 days.

One assay of this kit could prepare 1 mL BMDC differentiation medium (1 mL/ well in 24-well plate) and 0.5

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mL BMDC maturation medium. 200 assays can culture about 1×10^7 mouse bone marrow cells and harvest about $1\sim3\times10^7$ immature DC cells with 70-80% purity after 7 days of differentiation culture, and then can harvest about $1\sim3\times10^7$ mature DC cells with 80% purity after continue maturation culture for 24h.

Materials Not Supplied

- **Reagents**

  75% ethanol, RPMI 1640, Fetal Bovine Serum, Penicillin-streptomycin solution, L-alanyl-L-glutamine solution, Deionized water for cell culture, PBS for cell culture

- **Instruments**

  Centrifuge, CO_2 incubator, Inverted microscope, flow cytometer, Biosafety cabinet, Water bath, Pipettor

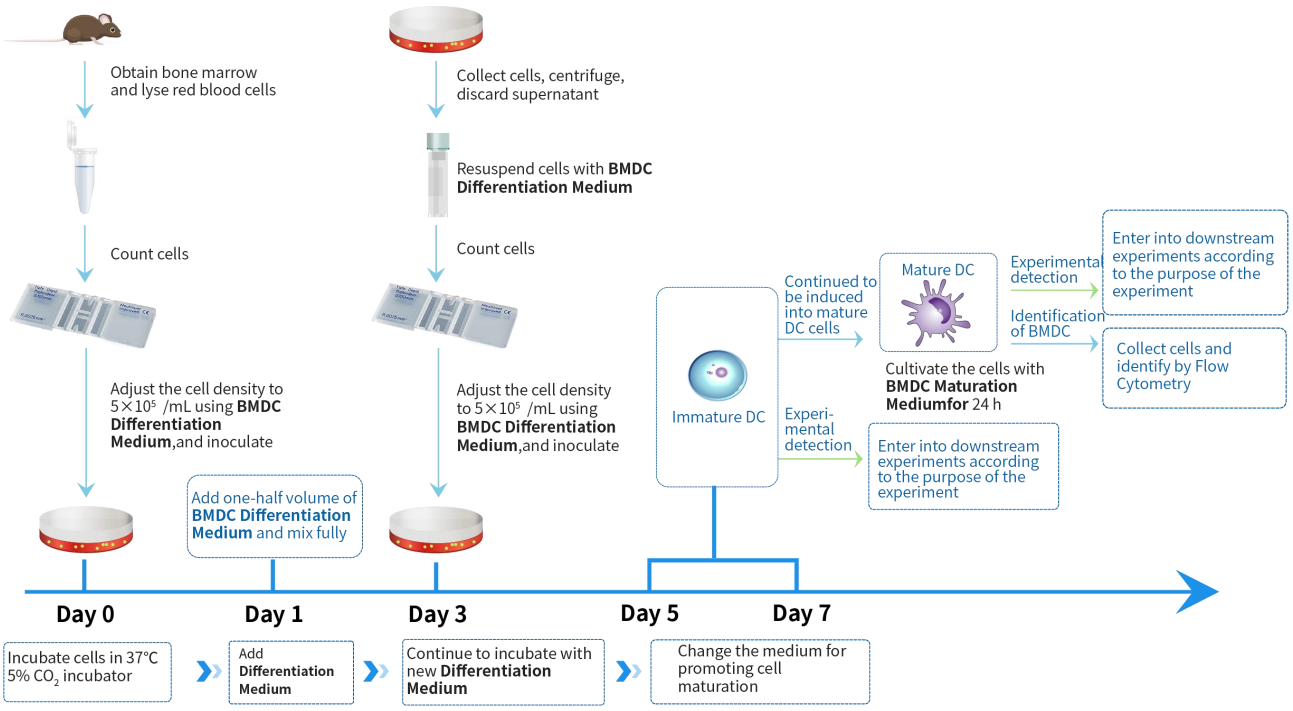
- **Materials**

  Petri dish, 300 mesh nylon filter membrane, 1 mL sterile syringe, ophthalmic scissors, ophthalmic forceps, sterile 15/50mL centrifuge tubes, pipette

Related Products

Products	Cat.	厂家
10×ACK Lysis Buffer	E-CK-A105	Elabscience
Cell Staining Buffer	E-CK-A107	Elabscience
RPMI 1640	PM150110	Pricella
L-Alanyl-L-Glutamine Solution	PB180419	Pricella
Penicillin-Streptomycin Solution	PB180120	Pricella

Assay Procedure



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Experimental Protocol

Note: Sterile technique is required for the isolation of BM cells.

➢ Reagent preparation

1) **BMDC Medium**: add L-alanyl-L-glutamine solution (final concentration of 2 mM), penicillin solution (final concentration of 100 U/mL) and streptomycin solution (final concentration of 100 μg/mL), and add fetal bovine serum (final concentration of 10%) to RPMI 1640 medium. The prepared BMDC Medium can be stored at 4℃ degrees for a month.

2) **BMDC Differentiation Medium**: Dilute Mouse DC Cell Differentiation MIX (1000×) to 1× with BMDC Medium. For example, add 50 μL Mouse DC Cell Differentiation MIX (1000×) into 50 mL BMDC Medium and mix fully. The prepared BMDC Differentiation Medium can be stored at 4℃ for 2 weeks.

3) **BMDC Maturation Medium**: Dilute Mouse DC Cell Maturation MIX (1000×) to 1× with BMDC Medium. For example, add 50 μL Mouse DC Cell Maturation MIX(1000×) into 50 mL BMDC Medium and mix fully. The prepared BMDC Maturation Medium can be stored at 4℃ for 2 weeks.

4) **Preparation of 1× ACK Lysis Buffer**: Remove the pre-cooled 10× ACK Lysis Buffer (pre-filtered with 0.22 μM filter membrane to remove bacteria) and sterile deionized water at 4°C, add 100 μL of 10× ACK Lysis Buffer to every 900 μL of sterile deionized water, gently mixture to make 1× ACK Lysis Buffer, 1 mL of 1× ACK Lysis Buffer was prepared for each mouse bone marrow cells.

➢ The isolation of mouse bone marrow cells

1) Mice were executed by appropriate methods, soaking in 75% ethanol for 5 minutes, take the femur and tibia bilaterally and remove excess muscle and other tissues under aseptic conditions. Be careful not to damage bones.

2) Place the femur and tibia in a Petri dish containing sterile PBS or RPMI 1640 medium, rinse twice, then transfer to a new Petri dish containing RPMI 1640 medium.

3) Cut off the epiphyses ends to both sides, use 1 mL sterile syringe (self-prepared) to pipette RPMI 1640 medium, insert the needle into the bone marrow cavity, gently rinse out the bone marrow into the Petri dish, aspirate new medium and repeat rinsing the bone marrow cavity for 2~3 times until the bone marrow cavity becomes completely white.

4) Use 1 mL syringe to aspirate cell clumps from the cell suspension and gently blow 2~3 times to fully dissociate the agglomerated cells.

   Note: Avoid cell damage caused by excessive blow operations..

5) Filter the above single-cell suspension with sterile 300 mesh nylon filter membrane, collect the cell suspension into a 15 mL centrifuge tube, then centrifuge at 150 g for 3 min, carefully discard the supernatant.

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6) Resuspend the bone marrow cells from each mouse with 1 mL of pre-cooled 1× ACK Lysis Buffer (red blood cell lysis, self-prepared) at 4°C, mix fully gently, and lysed for 0.5~1 min at room temperature.

7) Quickly add 10 mL sterile PBS or RPMI 1640 medium, terminate erythrocyte lysis by gently blowing and mix, centrifuge at 150 g for 3 min and carefully discard the supernatant.

8) Add 5 mL of BMDC Medium, resuspend the cell precipitate, centrifuge at 150 g for 3 minutes, and carefully discard the supernatant.

9) Add 10 mL of BMDC Differentiation Medium, resuspend the cells and count the cells.

Note: Approximately 1~3×10^7 bone marrow cells are obtained from each mouse.

➢ Differentiation culture of BMDCs

1) **Day0：** Adjust the cell density to 5×10^5/mL using BMDC Differentiation Medium, inoculate the cells into cell culture dishes, and incubate them in a 37℃ 5% CO_2 incubator.

2) **Day1：** Add one-half volume of BMDC differentiation medium and mix fully, observe the cell status under the microscope and take pictures.

3) **Day3：** Collect the suspended and loosely adherent cells, centrifuge at 150g for 3 min, carefully discard the supernatant, resuspend the cells using BMDC Differentiation Medium, adjust the cell density to 5×10^5/mL, inoculate the cells into a new cell culture dish, and continue culture in a 37 ℃ 5% CO_2 incubator.

4) **Day5:** Collect suspended cells and loosely adherent cells, count the cells, then centrifuge at 150g for 3 min, carefully discard the supernatant, resuspend the cells using BMDC Differentiation Medium, and adjust the cell density to 5×10^5/mL, inoculate the cells into new cell culture dishes, and then culture the cells in a 37℃ 5% CO_2 incubator, or conduct subsequent experiments as needed.

5) Cells have been differentiated into immature DC cells after 5~7 days of differentiation culture. Depending on the purpose of the experiment, cells can be collected for counting, entered into downstream experiments or continued to be induced into mature DC cells.

Note: During the 5th~7th day, the cell proliferation is very fast. It should be observed the density and status of cells at all times. If the color of the cell culture medium changes significantlyor a large number of cells aggregate and grow in clusters replenish one-half of the volume of the medium every 24h, and change the medium completely in 48h, so as to provide sufficient nutrition and growth environment for the cell proliferation. Prolonging the culture time is beneficial to increase the purity of the DC cells, and the purity of the DC cells can reach up to 70~80%.

➢ Maturation culture of BMDCs

1) Collect and count the suspended and loosely adherent cells on the 5th~7th day, centrifuge the cells at 150g for 3 min, carefully discard the supernatant, resuspend the cells using BMDC Maturation Medium, adjust the cell density to 5~8×10^5/mL, inoculate the cells into a new cell

culture dish, and continue to cultivate the cells for 24h at 37℃ in a 5%CO_2 incubator.

2) Collect suspended cells and loosely adherent cells, i.e., mature DC cells, count the cells for phenotypic characterization or conduct subsequent experiments as needed.

➢ Identification of BMDCs

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

1) Label the centrifuge tubes according to the table below, add 100 μL of immature or mature DC cells (1~5×10^5 cells) to each tube, add 1 mL of Cell Staining Buffer (self-prepared) or PBS buffer (self-prepared), mix fully gently, centrifuge at 150g for 3 min, discard the supernatant, add 100 μL of Cell Staining Buffer or PBS buffer to resuspend the cells.

2) Discard the supernatant, add 100 μL Cell Staining Buffer or PBS to resuspend the cell precipitate, add 2 μL of Purified Anti-Mouse CD16/32 Antibody to each group, mix fully gently, and incubate at room temperature with shading light for 10 min to close the FCR receptor on the surface of the cells, and then add the antibodies according to the table below, incubate at 4℃ for 30 minutes in the dark.

Purpose of the subgroups	Sample No.	Staining grouping
Voltage adjustment	1	Blank（blank cells, no antibodies）
Compensation adjustment (single-stained tube)	2	PerCP-MHC II
	3	Elab Fluor® 488-CD11c
	4	PE/Cyanine7-CD80
	5	PE-CD86
	6	APC-CD40
Full Panel	Isotype Control	PerCP-Rat IgG2b /ElabFluor®488-Armenian Hamster IgG / PE/Cyanine7- Armenian Hamster IgG/PE-Rat IgG2a/ APC-Rat IgG2a
	Test group	PerCP-MHC II/ElabFluor®488--CD11c/PE/Cyanine7-CD80/PE-CD86/ APC-CD40

Note: Antibodies used in the table can be found in the Relevant Product sections.

3) After incubation, add 1 mL of Cell Staining Buffer or PBS to each tube, mix fully gently, centrifuge at 150 g for 3 min, and discard the supernatant.

4) Resuspend the cells with 200 μL Cell Staining Buffer or PBS and detect with flow cytometer to and analyze.

Results Presentation

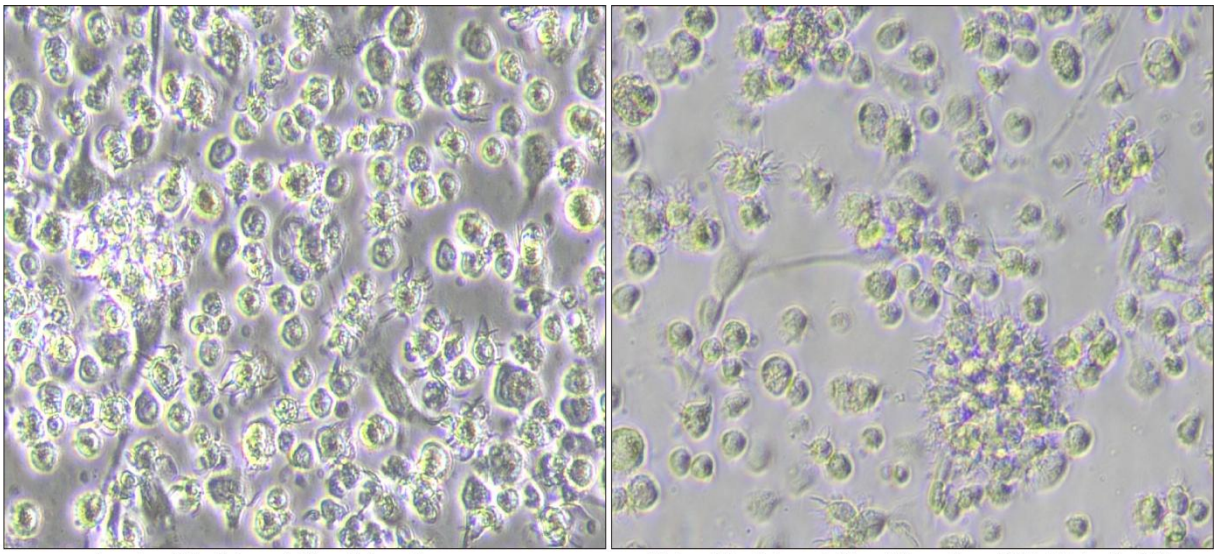

Figure 1. Morphological observation of BMDC: immature DC (left) cells have a few dendritic projections and few cells aggregate and grow in clusters; mature DC cells (right) have more dendritic projections and more cell clusters.

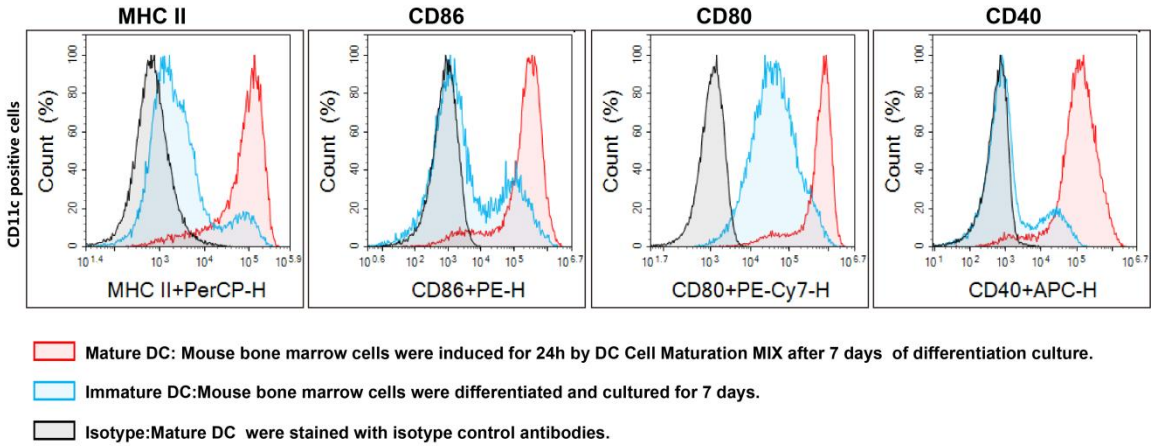

Figure 2. BMDC phenotyping: flow cytometry comparison of 7-week-old male C57/BL6 mice bone marrow cells differentiation culture for 7 days and 1-day post differentiation maturation promotion culture.

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. Approximately 1~3 × 10^7 bone marrow cells can be obtained from a 6~8 week old mouse. After 7 days of differentiation culture, approximately 3~7 × 10^7 immature DC cells with a purity of about 70~80% can be obtained.
4. It is recommended to select 6~8 weeks old healthy mice which have better activity of bone marrow cells to obtained better state of cultured DC cells. Since bone marrow cells of old mice have poorer differentiation ability, the DC cells obtained from the old one have lower purity and cell number.
5. The time of cell maturation should not be too long, 24h is the best. After maturation, the cells can be centrifuged and washed for relevant experiments to avoid cell death due to over-activation.
6. The time of erythrocyte lysis time is 0.5~1 min, should be strictly controlled not to exceed 1 min. Otherwise, excessive lysis will cause cell damage or loss. To ensure the harvest of more target cells, if there are fewer red blood cells and the cell precipitates into a grayish white color, erythrocyte lysis operation is not be essential.
7. To ensure effectiveness, the prepared cell differentiation and maturation promoting media should not be stored for a long time. It is recommended to use them up within 2 weeks.
8. Mouse DC Cell Differentiation MIX (1000×) and Mouse DC Cell Maturation MIX (1000×) can be stably stored at -20℃ for 1 year, if not used for a long time or need to be reused many times, it is recommended to store them at -80℃ and keep them away from light after dispensing, in order to ensure better stability and effectiveness of the reagents. If the reagent is not used for a long time or needs to be reused several times, it is recommended to aliquot the reagent into smaller quantities and store at -80°C
9. Please note the use of sterile red blood cell lysis.
10. 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.
11. Recommended Volumes of BMDC Medium for Various Cultureware:

Cultureware	Volume of BMDC Medium	Number of Cells/Well
96-well plate	100 µL/well	5×10^4
24-well plate	1 mL/well	5×10^5
12-well plate	2 mL/well	1×10^6
6-well plate	3 mL/well	1.5×10^6
60 mm Cell Culture Dishes	5 mL/well	2.5×10^6
100 mm Cell Culture Dishes	10 mL/well	5×10^6

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