ImmunoCult™ Dendritic Cell Culture Kit

For Differentiation of Human Monocytes into Dendritic Cells

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

ImmunoCult™ Dendritic Cell Culture Kit has been developed for the in vitro culture and differentiation of human monocytes into dendritic cells (DCs) and their subsequent maturation into fully active mature DCs. This kit enables the user to generate active mature DCs from human monocytes in 7 days, using an animal component-free (ACF) medium.

ImmunoCult™ Dendritic Cell Culture Kit (Catalog #10985) includes ImmunoCult™-ACF Dendritic Cell Medium (100 mL), ImmunoCult™-ACF Dendritic Cell Differentiation Supplement (1 mL), and ImmunoCult™ Dendritic Cell Maturation Supplement (0.5 mL).

ImmunoCult™-ACF Dendritic Cell Medium is a serum-free and animal component-free medium optimized for the in vitro culture and differentiation of human monocytes into DCs. This medium contains recombinant proteins and synthetic components. It does not contain serum or other human- or animal-derived components. Recombinant growth factors, required for the optimal differentiation and maturation of DCs, have not been added to ImmunoCult™-ACF Dendritic Cell Medium. This allows users the flexibility to prepare a medium that meets their requirements.

The ImmunoCult™-ACF Dendritic Cell Differentiation Supplement contains a combination of animal component-free recombinant human cytokines formulated to support the differentiation of DCs from human monocytes. It is supplied as a 100X concentrate.

The ImmunoCult™ Dendritic Cell Maturation Supplement is formulated to support the maturation of immature DCs. It is supplied as a 100X concentrate.

Product Information

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
ImmunoCult™-ACF Dendritic Cell Medium	10986	500 mL	Store at 2 - 8°C.	Stable for 18 months from date of manufacture (MFG) on label.
ImmunoCult™ Dendritic Cell Culture Kit	10985	1 Kit		
ImmunoCult™-ACF Dendritic Cell Medium	10987	100 mL	Store at 2 - 8°C.	Stable for 18 months from date of manufacture (MFG) on label.
ImmunoCult™-ACF Dendritic Cell Differentiation Supplement	10988	1 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
ImmunoCult™ Dendritic Cell Maturation Supplement	10989	0.5 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.

Preparation of ImmunoCult™ DC Differentiation Medium

Use sterile techniques to prepare ImmunoCult™ DC Differentiation Medium (ImmunoCult™-ACF Dendritic Cell Medium + ImmunoCult™-ACF Dendritic Cell Differentiation Supplement). The following example is for preparing 10 mL of medium. If preparing other volumes, adjust accordingly.

1. Thaw Differentiation Supplement at room temperature (15 - 25°C) or at 37°C until just thawed. Mix thoroughly.

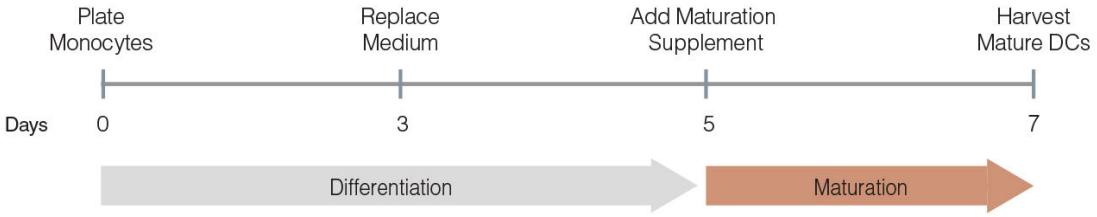
   NOTE: If necessary, centrifuge supplement for 30 seconds to collect liquid from cap.

   NOTE: If not used immediately, aliquot and store at 2 - 8°C for up to 1 month. Alternatively, store aliquots at -20°C. Do not exceed the shelf life of the supplement. After thawing aliquots, use immediately. Do not re-freeze.

2. Add 100 µL of Differentiation Supplement to 9.9 mL of Dendritic Cell Medium. Mix thoroughly.

   NOTE: If not used immediately, store ImmunoCult™ DC Differentiation Medium at 2 - 8°C for up to 3 days.

Protocol Diagram



Directions for Use

Please read the entire protocol before proceeding.

The following instructions are for use with a T-25 cm^2 flask. If using alternative cultureware, adjust volumes accordingly.

1. Isolate monocytes from fresh (< 24 hours old) human whole blood or from leukapheresis samples using an EasySep™ negative selection cell separation kit.

   NOTE: For optimal cell yield in this application, we recommend using the EasySep™ Human Monocyte Isolation Kit (Catalog #19359). For other EasySep™ kits that may be used, see Notes and Tips.

2. If using a T-25 cm^2 flask, add purified human monocytes at 1 x 10^6 cells/mL to 5 mL of ImmunoCult™ DC Differentiation Medium (see Preparation section). Refer to Table 1 for volumes required for other types of cultureware.

   Table 1. Recommended Volumes of ImmunoCult™ DC Differentiation Medium for Various Cultureware

CULTUREWARE	VOLUME OF IMMUNOCULT™ DC DIFFERENTIATION MEDIUM	NUMBER OF CELLS/WELL
96-well plate	100 µL/well	1 x 10^5
24-well plate	500 µL/well	5 x 10^5
12-well plate	1 mL/well	1 x 10^6
6-well plate	2.5 mL/well	2.5 x 10^6

3. Add the cell suspension (5 mL) to a T-25 cm^2 flask. Incubate at 37°C for 3 days.
4. **Day 3**: Remove the medium by pipetting from the flask and add to a 14 mL centrifuge tube. Quickly, add 5 mL of fresh ImmunoCult™ DC Differentiation Medium to the culture flask.
5. Centrifuge the 14 mL tube containing medium and cells (from step 4) at 300 x *g* for 10 minutes. Remove and discard the supernatant. Resuspend cells in a small volume (i.e. 50 µL or up to 10% of the original volume) of fresh ImmunoCult™ DC Differentiation Medium and return to the culture flask.

   NOTE: This step saves non-adherent or loosely adherent cells.

6. Incubate at 37°C for 2 days.
7. **Day 5**: Add ImmunoCult™ Dendritic Cell Maturation Supplement directly to the culture flask at a 100X dilution (e.g. add 50 µL Maturation Supplement to approximately 5 mL culture medium). Swirl gently to mix. Do not change medium.
8. **Day 7**: Harvest fully mature DCs by gently pipetting up and down to ensure all cells are in suspension, then transfer to an appropriate tube.

Notes and Tips

In addition to the EasySep™ Human Monocyte Isolation Kit (Catalog #19359), other EasySep™ kits that may be used include:

- EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion (Catalog #19058)
- EasySep™ Direct Human Monocyte Isolation Kit (Catalog #19669)

For phenotype assessment of mature DCs (CD14-CD83+) by flow cytometry use the following fluorochrome-conjugated antibodies:

- Anti-Human CD14 Antibody, Clone MoP9 (Catalog #60124) and Anti-Human CD83 Antibody, Clone HB15e (Catalog #60107)

NOTE: To minimize non-specific binding, we recommend using Anti-Human CD32 Antibody, Clone IV.3 (Catalog #60012) as an FcR blocker. If labeling mature DCs with Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004), use Mouse IgG2a, kappa Isotype Control Antibody, Clone MOPC-173 (Catalog #60071) or rat serum in addition to Anti-Human CD32 Antibody, Clone IV.3.