

# ImmunoCult™-SF Macrophage Medium

**Serum-free medium for differentiation of human monocytes to macrophages**

Catalog #10961      250 mL



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## Product Description

ImmunoCult™-SF Macrophage Medium has been developed for the in vitro culture and differentiation of human monocytes into macrophages when the appropriate cytokines and stimuli are added. This medium is serum-free and contains pre-tested bovine serum albumin, insulin, transferrin, and other supplements in Iscove's MDM. The factors for differentiation and activation of macrophages have not been added to ImmunoCult™-SF Macrophage Medium. This provides users the flexibility to prepare medium that meets their requirements. When the appropriate cytokines and stimuli are added, ImmunoCult™-SF Macrophage Medium supports the differentiation of human monocytes into M1 (classically activated) or M2 (alternatively activated) macrophages in a 6- or 8-day culture period.

## Product Information

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
ImmunoCult™-SF Macrophage Medium*	10961	250 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

\*This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

## Preparation of ImmunoCult™-SF Macrophage Differentiation Medium

Use sterile techniques to prepare ImmunoCult™-SF Macrophage Differentiation Medium (ImmunoCult™-SF Macrophage Medium + Human Recombinant M-CSF [Catalog #78057]). The following example is for preparing 10 mL of medium. If preparing other volumes, adjust accordingly.

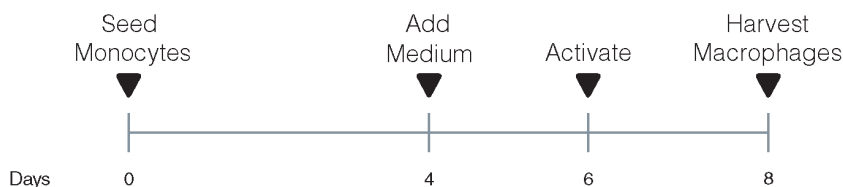
1. Thaw ImmunoCult™-SF Macrophage Medium at room temperature (15 - 25°C) until just thawed. Mix thoroughly.  
NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the shelf life of the medium. After thawing aliquots, use immediately or store at 2 - 8°C for up to 4 weeks. Do not re-freeze.
2. Add 100 µL of 5 µg/mL Human Recombinant M-CSF to 10 mL of ImmunoCult™-SF Macrophage Medium (final concentration 50 ng/mL). Mix thoroughly.  
NOTE: If not used immediately, store ImmunoCult™-SF Macrophage Differentiation Medium at 2 - 8°C for up to 1 week.

## Protocol Diagrams

### 6-Day Culture Protocol



### 8-Day Culture Protocol



## Directions for Use

Please read the entire protocol before proceeding.

The following instructions are for the culture and differentiation of human monocytes into M1 or M2a macrophages using a 6- or 8-day culture protocol. For other macrophage subtypes, add appropriate stimuli; protocol may need to be optimized.

The following instructions are for a T-25 cm<sup>2</sup> flask. If using alternative cultureware, refer to Table 1 and adjust cell numbers and volumes accordingly.

### Day 0

- 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, we recommend using EasySep™ Human Monocyte Isolation Kit (Catalog #19359). For other compatible EasySep™ kits, see Notes and Tips.
- If using a T-25 cm<sup>2</sup> flask, add purified human monocytes at  $1 \times 10^6$  cells/mL to 5 mL of ImmunoCult™-SF Macrophage Differentiation Medium (see Preparation section). Refer to Table 1 for other cultureware.

**Table 1. Recommended Volumes and Cell Numbers for Various Cultureware**

CULTUREWARE	DAY 0		DAY 6/DAY 8	
	VOLUME OF IMMUNOCULT™-SF MACROPHAGE DIFFERENTIATION MEDIUM	NUMBER OF CELLS/WELL	VOLUME OF DISSOCIATION REAGENT	VOLUME OF PBS + 0.5% BSA
96-well plate	100 µL/well	$1 \times 10^5$	100 µL/well	150 µL/well
24-well plate	500 µL/well	$5 \times 10^5$	500 µL/well	1 mL/well
12-well plate	1 mL/well	$1 \times 10^6$	1 mL/well	2 mL/well
6-well plate	2.5 mL/well	$2.5 \times 10^6$	2.5 mL/well	5 mL/well

PBS: phosphate-buffered saline; BSA: bovine serum albumin

- Add the cell suspension to the T-25 cm<sup>2</sup> flask. Incubate at 37°C for 4 days.

### Day 4

- Add 2.5 mL (half of the original volume) of fresh ImmunoCult™-SF Macrophage Differentiation Medium to the flask.

NOTE: Alternatively, this medium addition may be done on Day 3 instead of Day 4.

- For a **6-day culture period**, continue to step 6 for activation.

OR

For an **8-day culture period**, incubate at 37°C for 2 days, then proceed to step 6 for activation.

**Day 4/Day 6**

6. Activate the culture as follows:
- **M1 activation:** Add 10 ng/mL LPS and 50 ng/mL IFN- $\gamma$  (Catalog #78020)

OR

- **M2a activation:** Add 10 ng/mL IL-4 (Catalog #78045)

7. Incubate at 37°C for 2 days.

**Day 6/Day 8**

8. Harvest macrophages as follows:

- Remove supernatant from flask and transfer to a 50 mL conical tube.
- OPTIONAL (for ELISA): Centrifuge the tube at 300 x *g* for 10 minutes. Remove supernatant and retain for ELISA. Retain cell pellet.
- Add dissociation reagent to the flask as follows (see Table 1 if using other cultureware):
  - For **M1 macrophages:** Add 5 mL of ACCUTASE™ (Catalog #07920)OR
  - For **M2a macrophages:** Add 5 mL of 2.5 mM EDTA in phosphate-buffered saline (PBS)
- Incubate at 37°C for 15 minutes.
- Add 10 mL of PBS + 0.5% bovine serum albumin (BSA) (see Table 1 if using other cultureware).
- Pipette the cells up and down several times.
- Transfer cell suspension to the tube containing culture supernatant (step a) or cell pellet (step b).
- Centrifuge tube at 300 x *g* for 10 minutes. Remove and discard supernatant.
- Resuspend macrophages in appropriate medium or buffer for downstream applications.

## Notes and Tips

- In addition to EasySep™ Monocyte Isolation Kit (Catalog #19359), other EasySep™ kits that can be used include:
  - EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion (Catalog #19058)
  - EasySep™ Direct Human Monocyte Isolation Kit (Catalog #19669)
- For phenotypic assessment of macrophages by flow cytometry, we recommend the following fluorochrome-conjugated antibodies:
  - Anti-human CD80 antibody, clone 2D10
  - Anti-human CCR7 antibody, clone G043H7
  - Anti-human CD206 antibody, clone 15-2
  - Anti-human CD209 antibody, clone 9E9A8
  - Anti-Human CD14 Antibody, Clone MoP9 (Catalog #60124) or Clone M5E2 (Catalog #60004)
- To minimize non-specific binding, use Anti-Human CD32 Antibody, Clone IV.3 (Catalog #60012) as an FcR blocker, in combination with rat serum

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