# ImmunoCult™-SF Macrophage Medium

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

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Catalog #10961 250 mL

## 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.

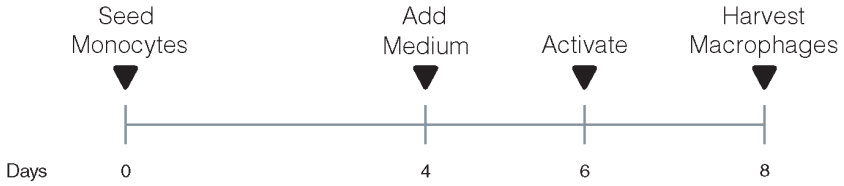
2. Add 100 µL of 5 µg/mL Human Recombinant M-CSF to 10 mL of ImmunoCult™-SF Macrophage Medium (final concentration 50 ng/mL). Mix thoroughly.

   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 $cm^2$ flask. If using alternative cultureware, refer to Table 1 and adjust cell numbers and volumes accordingly.

### Day 0

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, we recommend using EasySep™ Human Monocyte Isolation Kit (Catalog #19359). For other compatible EasySep™ kits, 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™-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 x 10^5 | 100 µL/well | 150 µL/well |
| 24-well plate | 500 µL/well | 5 x 10^5 | 500 µL/well | 1 mL/well |
| 12-well plate | 1 mL/well | 1 x 10^6 | 1 mL/well | 2 mL/well |
| 6-well plate | 2.5 mL/well | 2.5 x 10^6 | 2.5 mL/well | 5 mL/well |

3. Add the cell suspension to the T-25 $cm^2$ flask. Incubate at 37°C for 4 days.

### Day 4

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.

5. 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-γ (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:
   a. Remove supernatant from flask and transfer to a 50 mL conical tube.
   b. OPTIONAL (for ELISA): Centrifuge the tube at 300 x *g* for 10 minutes. Remove supernatant and retain for ELISA. Retain cell pellet.
   c. 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 PBS
   d. Incubate at 37°C for 15 minutes.
   e. Add 10 mL of PBS + 0.5% BSA (see Table 1 if using other cultureware).
   f. Pipette the cells up and down several times.
   g. Transfer cell suspension to the tube containing culture supernatant (step a) or cell pellet (step b).
   h. Centrifuge tube at 300 x *g* for 10 minutes. Remove and discard supernatant.
   i. 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