# DIFFERENTIATION OF CD14+ MONOCYTE INTO M1-/M2- MACROPHAGES

The Human CD14+ Monocytes (T4122) is isolated from human peripheral blood and can differentiate into M1-Macrophages or M2-Macrophages using **abm**'s M1-Macrophage Differentiation Medium (TM006) or M2-Macrophage Differentiation Medium (TM007), respectively. Both M1-/M2- Macrophage Differentiation Medium are complete medium containing all growth factors and supplements necessary for efficient generation of monocyte-derived macrophages.

**Protocol**

1. Thaw and plate Human CD14+ Monocytes using PriGrow II (TM002) supplemented with 10% heat-inactivated fetal bovine serum and Penicillin/Streptomycin (G255). Incubate plate for 24 hours at 37°C and 5% $CO_2$.

2. Replace the culture medium with appropriate volume of Macrophage Differentiation Medium (see Table 1 below). Incubate plate for 7 days at 37°C and 5% $CO_2$.

3. After 7 days, add another 50% volume of fresh Macrophage Differentiation Medium and incubate the cells for another 3 days at 37°C and 5% $CO_2$.

4. Supplement the medium directly with activation factors to activate the macrophages. Classic activation factors for M1-macrophages include IFN-γ (50ng/ml) and LPS (10ng/ml) while M2-macrophages use IL-4 (20ng/ml). Harvest macrophages 24 hours after addition of stimulating agent for experiments.

**Table 1: Volume of Macrophage Differentiation Medium for Different Culture Vessels**

| Culture Wares | Area ($cm^2$) | Volume |
|---|---|---|
| 96- well plates | 0.143 | 0.15 ml/ well |
| 24- well plates | 0.33 | 1.0 ml/ well |
| 12- well plates | 1.12 | 2.0 ml/ well |
| 6- well plates | 4.67 | 3.0 ml/ well |
| T-25 flask | 25 | 7.0 ml/ flask |
| T-75 flask | 75 | 20.0 ml/ flask |

*For laboratory research only. Not for clinical applications.*

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