Passaging Blood or Bone Marrow Derived Macrophages
Using Accutase Cell Detachment Solution

Primary macrophage cultures may be derived from density gradient separated whole blood or bone marrow by culturing these isolates in the presence of specific growth factors to encourage the growth, expansion, and differentiation of the macrophage cell type. A literature search will yield several in-depth protocols which out-line these isolation techniques in detail. Once harvested and put into culture at 37°C in a 5% CO_2 incubator the macrophage progenitor cells will adhere to tissue culture flasks and will not be washed away when the media is changed to remove any non-adherent cells and allow for macrophage differentiation/maturation.

To remove the Primary macrophage cultured cells from the tissue culture plates for further analysis:

1. Remove the supernatant from the flask (T25) or tissue culture dish (15 mm).
2. Wash the plate or dish with 5 ml sterile PBS and remove it.
3. Add 5 ml of Accutase to the flask or dish and incubate at room temperature for 10-15 minutes. Inspect the flask under the inverted microscope to look for cell "shrinkage" or detachment.
4. Gently swirl the flask and add another 5 ml of Accutase. Incubate the cells another 10-15 minutes at room temperature. Resuspend the cells by trituration (pipetting up and down several times) being careful not to cause bubbles.
5. Count the cells and adjust the concentration to be used for further analysis.

NOTE: This procedure may also be carried out on ice which may decrease the incubation times needed.

Passaging Macrophage Cell Lines such as DH82 and RAW264.7
Using Accutase Cell Detachment Solution

For those investigators who prefer to utilize immortalized cell lines rather than isolate primary cultures, Accutase may be used to passage their macrophage lines in a similar manner to the techniques used for most other adherent cell lines.

1. Remove flask (T25) from the incubator and examine it under the inverted microscope to check for cell confluency.
2. Transfer the flask to the tissue culture hood for cell passaging. Remove the media from the flask. Wash the flask 2 times with 5 ml of sterile PBS; fully remove after each addition.
3. Add 5 ml of Accutase to the flask and incubate it at room temperature for 5-10 minutes.
4. Inspect under the microscope for classic signs of cell detachment i.e., "shrinkage" or "rounding".
    a. If there are significant signs of "rounding", then transfer the plate back to the hood and triturate (pipette up and down several times) being careful to not create bubbles.
    b. If cells do not appear significantly "rounded" then allow them to incubate and additional 2-3 minutes and proceed as in step "a" above.
5. Passage cells at a 1:2 or 1:4 ratio every 3-6 days.

Please note that different adherent cells stick to tissue culture plastic with varying degrees of adherence. For this reason, the incubation time required for detachment can vary with some cell types needing more or less time to detach. In the case of extremely tenaciously adherent cells our stronger formulation, Accumax may work better.