Passaging Blood or Bone Marrow-derived Macrophages Using Accutase® Cell Detachment Solution

Protocol

CORNING

Accutase® is an effective solution for routinely detaching cells from standard tissue culture-treated vessels, as well as advanced surface treatments or coatings. Accutase is useful for cell detachment and for preparing single-cell suspensions from clumped cell populations for sub-culturing cells, analytical studies, and for accurate cell counting. Accutase is free of mammalian or bacterial-derived products, which reduces the risk of contamination.

Accutase is formulated at a concentration that is ready to use, once thawed. **(Note: Never thaw Accutase at 37°C.)** A thawed bottle of Accutase can be removed from the refrigerator and immediately applied to cells. **It should not be pre-warmed to 37°C.** Accutase contains proteolytic activity that gently breaks down cellular adhesion molecules and enables cell detachment from the bottom of the flask.

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. An online search will yield several in-depth protocols that outline 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 in order 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. Transfer the flask to the tissue culture hood. Remove the media from the flask (T-25) or tissue culture dish (15 mm).
2. Wash the plate or dish with 5 mL sterile Phosphate-Buffered Saline (PBS) and discard the saline solution.
3. Add 5 mL of Accutase® (Corning Cat. No. 25-058-CI) to the flask or dish and incubate at room temperature for 10 to 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 to 15 minutes at room temperature. Resuspend the cells by pipetting up and down several times being careful not to cause bubbles.
5. Centrifuge in conical tube containing the cell suspension at 200 x g for 4 minutes. Remove media.
6. 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

1. 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 the PBS after each addition.
2. Add 5 mL of Accutase to the flask and incubate it at room temperature for 5 to 10 minutes.
3. 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 pipette up and down several times, being careful to not cause bubbles.
   b. If cells do not appear significantly "rounded," then allow them to incubate an additional 2 to 3 minutes and proceed as in Step "a" above.
4. Passage cells at a 1:2 or 1:4 ratio every 3 to 6 days.

Please note that different adherent cells stick to tissue culture surfaces with varying degrees of adherence. For this reason, the incubation time required for detachment can vary. Some cell types will need more, or less, time to detach.

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At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.

It is this expertise, plus a 160-year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high-quality, reliable cell culture consumables.

For additional product or technical information, please visit www.corning.com/lifesciences/media or call 1.800.235.5476.

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