



TIPS FOR SUCCESSFUL SUB-CULTURING: DISSOCIATION METHOD

TAILORING YOUR DISSOCIATION METHOD TO YOUR CELL LINE.

There are many parameters to consider when developing the culture conditions for a particular cell line. We all know that pH balance, oxygen levels, and serum concentration are important variables that contribute to the success or failure of the culture. However, a sub-culturing protocol that includes a dissociation method matched to the needs of the cell is just as important. With that in mind, we will review the principles behind some common dissociation practices, to help you tailor your methods to the specific nature of your cell line.

Cells that grow in suspension are easy to sub-culture. They will do fine as long as the cells are diluted into suitable, fresh media before they use up their nutrient supply. Cells that grow in monolayers, on the other hand, develop proteinaceous contacts with each other and with the culture dish. These must be broken before the cells can be suspended in culture media, spun down, and re-plated. Therefore, special considerations must be made to ensure the dissociation conditions disrupt cell contacts without killing the cell.

Trypsin is sometimes, but not always, the answer

When deciding on the dissociation conditions, the investigator should take into account the type of bonds made by the cells. Some adherent cell lines make only weak contacts that can be disrupted by simply striking the culture flask against the palm of your hand, or by tapping the flask against the counter-top. Other adherent cell lines, particularly those derived from an epithelium, make strong, multi-protein tight junctions that can only be disrupted by enzymatic digestion; in these cases, trypsin (a serine protease) is often employed. Cells derived from an epithelium, may also express cadherin proteins, which mediate very strong calcium-dependent adherens or desmosomal junctions. In such cases, the addition of a calcium chelator (like EDTA), which sequesters calcium, may be necessary to dissociate the cells effectively.

Optimizing viability after dissociation

To optimize cell viability after dissociation, the reaction conditions should match the strength of the cell contacts. For example, if standard trypsin/EDTA digestion (usually 0.05%-0.5%) does not efficiently digest cell bonds (within 10-15 minutes) it may be difficult

to remove the cells from the plate before they start to die, and cell viability will be adversely affected. In this case, a higher concentration of trypsin/EDTA or additional enzymes, such as collagenase, may be needed. On the other hand, if the reaction is too harsh, the cells may lyse or lose surface proteins necessary for reattaching to the plate, and either scenario will lead to reduced viability. In addition, lysed cells release genomic DNA, which causes viable cells to clump, making the surviving cells harder to re-plate, and necessitating the addition of DNase.

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Preparation is key to good dissociation

If you have matched the strength of the reaction to the adherence properties of the cell line, but your cells are still difficult to remove, you may find that the problem lies with the way the cells were prepared for dissociation. There may be elements, such as serum, in the growth media that are inhibiting the trypsin, but this can be overcome by simply rinsing the cells once or twice with Dulbecco's PBS (without calcium or magnesium) before adding the dissociation reagents. It may also be that the cells were allowed to remain confluent for too long. Under these conditions, cells may develop exceptionally strong cell-cell and cell-surface junctions that are exceptionally difficult to break. For this reason, as well as for the overall health of the culture, it is recommended that the cells be passaged before they become 100% confluent (i.e., 70-90% confluent).

Conclusion

Armed with a basic understanding of how dissociation reagents work, investigators can develop an optimized protocol that takes both the strength and quality of the cell's adherence properties into consideration. An optimized protocol for the dissociation of your cells will ensure successful sub-culturing and prolong the life of your cells in culture.

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p 800.638.6597
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e tech@atcc.org
www.atcc.org

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