Choosing Optimal Harvesting Solutions

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There are many reagents available for harvesting anchorage-dependent cells. Options include solutions that are animal-derived, recombinant or fully synthetic. Additionally, the mechanisms by which the reagents work varies. Some work via enzymes such as proteases or collagenases, while other options can be completely non-enzymatic and act via chelation of ions that aid in cell interaction with plastic. The most appropriate harvesting solution should consider the biology of the cell and further downstream applications. Although some harvesting reagents are more common than others, each can impact cell recovery, viability, and functionality. In this article, we highlight commonly used harvesting reagents and their advantages.

Trypsin

Trypsin is one of the most used enzymes for harvesting cells due to its robust proteolytic enzyme activity and ease to manufacture. Trypsin is animal-derived as it is obtained from mammalian digestive tracks. While different cell types can require different concentrations, the typical concentration used to harvest tissue culture is between 0.025% to 0.05% (v/w), and it is often supplemented with EDTA.

Trypsin works by cleaving bonds in proteins and peptides containing a carboxyl group of lysine or arginine[1] with optimal digestion performance at 37°C. Following the release of the cells during harvest, further Trypsin activity can be halted by adding solutions containing excess serum proteins (such as complete growth medium)[2]. This step, often called inactivation or neutralization, can prevent over-digestion of cells. Thus, harvesting times and concentrations of Trypsin used should be optimized to find the most appropriate conditions. For situations where serum-free medium is being used, soybean inhibitor can be a suitable neutralizing alternative. Because Trypsin doesn't specifically cleave extracellular matrix proteins, it is possible to over-digest and damage cells or cleave receptors that are important for downstream applications. Care needs to be taken to optimize harvesting protocols with Trypsin.

NOTE: Because Trypsin doesn't specifically cleave extracellular matrix proteins, it is possible to over-digest and damage cells or cleave receptors that are important for downstream applications. This is especially important when harvesting cells for screening molecules.

Accutase®

Accutase is an alternative enzyme mixture formulated with proteolytic and collagenolytic enzyme activity. Accutase solution does not contain mammalian or bacterial-derived products and is typically considered gentler than Trypsin. Because of its gentler enzymatic and collagenolytic activity, Accutase is ideal for applications where intact surface markers are required and for single cell passaging of stem cell cultures[3]. Additionally, Accutase does not require inactivation like Trypsin, so it works well for serum-free applications by simply diluting Accutase to prevent further digestion.

NOTE: Accutase has been shown to work well in situations where cells secrete a heavy matrix coat.

Dispase

Dispase is a protease enzyme isolated from bacteria. It cleaves fibronectin and Type IV Collagen, but not Laminin or Type V Collagen[4]. The specificity of Dispase makes it ideal for certain applications but ineffective for others. Since Type IV Collagen is one of the main proteins of the basement membrane, Dispase can be an effective tool for breaking down primary tissue explants[5]. Alternatively, when used alone (at concentrations between 0.025 to 0.6 U/mL), it will not readily achieve single cell suspensions from a typical monolayer culture.

NOTE: Dispase is also not inactivated by proteins found in serum and must be inactivated by the addition of chelators, such as EDTA or EGTA. It is also recommended to centrifuge in order to remove Dispase, as it can be toxic to some cells[6].

Corning Cell Recovery Solution

Corning cell recovery solution is a non-enzymatic, proprietary solution that can be used to recover embedded cells from Corning® Matrigel® matrix. This solution can depolymerize a 1 mm thick layer of gelled Matrigel matrix after one hour at 2°C to 8°C. Freeing cells or spheroids from Matrigel matrix can be helpful for staining or other downstream applications. Cell recovery solution will not break up spheroids or impact cellular viability. If further digestion of cells is required, other harvesting solutions should be used after recovering cells from Matrigel matrix.

NOTE: For maximal effectiveness, Corning cell recovery solution should be added at a ratio of at least two times the volume of Matrigel matrix.

Corning Cellstripper®

Corning Cellstripper is a non-enzymatic cell dissociation solution, formulated with a proprietary mixture of chelators, which gently dislodges adherent cells in culture. It is an ideal solution when cells need to be harvested as gently as possible or without serum. Since there are no proteolytic or collagenolytic enzymes present, Cellstripper is suitable for cells that do not generate a lot of ECM. Additionally, there is no inactivation required. Once cells are harvested, they can be collected in medium and used immediately.

Key Features

Cat. No.	Harvesting Reagent	Animal-derived	Inactivation Requirement	Mechanism
25-051-CI 25-050-CI 25-054-CI 25-052-CV 25-052-CI	Trypsin	Porcine-derived	Serum or soybean inhibitor	Enzymatic
25-058-CI	Accutase®	Marine origin	Dilution	Enzymatic
354235	Dispase	Bacteria-derived	Chelators such as EDTA	Enzymatic
354253 354270	Cell Recovery Solution	No	Not required	Non-enzymatic
25-056-CI	Corning® Cellstripper®	No	Not required	Non-enzymatic

References

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