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TECH NOTE

Non-enzymatic Methods for Cell Harvesting

In some cases it is necessary to harvest cells culture vessels without using protein digesting enzymes, proteases, such as trypsin, collagenase and pronase. These proteases may modify cellular proteins or cell membranes, thus affecting some immunological or physiological assays. Non-enzymatic dissociation solutions with EDTA (ethylenediaminetetraacetic acid) and mechanical methods like scraping and freezing are good alternatives for most purposes.

Non-enzymatic Cell Dissociation Solutions

EDTA diluted to 0.1-2.0% in either Hank's Buffered Saline Solution (HBSS) (1) or Phosphate Buffered Saline (PBS) (2) chelates calcium and magnesium from intercellular bridges. Cell dissociate from each other as well as from plastic and glass. The HBSS and PBS used for dilution must be Ca^{++} and Mg^{++} free. Different cell lines may require different concentrations of EDTA and various incubation times. The addition of a complete growth medium or buffer containing balanced calcium and magnesium reconstitutes cells for re-seeding or assay procedures.

Sigma Chemical Company (St. Louis, MO; 800-325-8070 in the U.S.A. and Canada or collect 314-771-5765 outside the U.S.A. and Canada) has two products specifically formulated for non-enzymatic cell dissociation. Cell Dissociation Solution, Non-enzymatic in HBSS (C5789) or PBS (C5914) contains EDTA, glycerol and sodium citrate.*

Sigma claims that the solutions work faster than protease solutions, yet are gentle, resulting in 70-100% viability for various cell lines. As viability is dependent on how cells are handled and on the laboratory technique employed, these solutions are good alternatives when enzymes are undesirable.

* Mention of the Sigma product does not constitute an endorsement or a specific recommendation for use of this product. All technical questions must be directed to Sigma Chemical Company.

The following general protecol is suggested for EDTA based solutions:

1. Pre-warm all reagents to 37°C.
2. Remove medium from the culture vessel without drying the monolayer.
3. Rinse the cells with a balanced salt solution without calcium or magnesium. Gently rock the vessel for 30 seconds, then remove the buffer.
4. Add the cell dissociation solution (about 5 ml/75 cm^2 flask, 30 ml/TripleFlask and 30 ml/Cell Factory tray). Rock the vessel to bathe the cell monolayer.
5. Incubate 5-10 minutes. Strongly adherent cells may require additional time to become dislodged.
6. Shake the vessel or tap sharply against the palm of your hand to dislodge the cells.
7. Pour the cell suspension from the vessel to collect. Rinse the vessel with a balanced salt solution or complete growth medium as necessary and add to initial collection.

Some cell types may tend to come off in sheets or may have a tendency to clump. In most cases, shaking or rocking the vessel will break up the cell mass sufficiently to permit pouring the cells into a test tube or small bottle. Pre-warming all reagents and rinsing monolayers with Ca^{++}/Mg^{++} free HBSS or PBS can decrease incubation times and make dissociation more efficient.

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Mechanical Methods

Scraping

Scraping the culture flask in a pre-warmed, balanced salt solution, then dissociating the cells by gentle vortexing or trituration (repeated, gentle pipetting) results in viable and intact cells. The addition of 5-10% serum may be necessary to ensure recovery of some delicate cells.

Freezing

Freezing is another alternative for cell harvesting. Although cells are not usually viable after freezing, many membrane characteristics and immunological properties remain intact. This technique is often used for harvesting protease sensitive, biological materials rather than whole cells.

Remove medium from culture vessel. To prevent the monolayer from drying, immediately add a balanced salt solution to the vessel (5-10 ml/75 cm^2 flask, 30-50 ml/Triple Flask). Complete growth medium with 5-20% serum, or alternatively, a cell freezing medium may be necessary for some procedures. Freeze the vessel at –20°C until the harvest solution i completely frozen.

While Nunc has thoroughly tested freezing techniques in T.C. Flasks, several investigators have used cryo-harvesting under production conditions to isolate materials from the Cell Factory. Their exact protocols vary slightly; however, their procedures use 50-100 ml buffer or medium per tray, following the above suggested guidelines.

Expansion of the buffer or medium may cause the vessel to crack so use the minimum volume necessary and leave the caps loose or freeze with a Filter Cap. Mount filters on Cell Factory ports.

Polystyrene is an insulator so freezing is slow in multilayered vessels. Although time may be adjusted by the individual researcher, we find that at least two hours at –20°C after the solution is completely frozen, assures uniform dispersion. Do not freeze culture vessels at extreme temperatures (i.e. –80°C).

Care must be taken when handling frozen vessels. Do not jar or rap frozen vessel. Support multilayer vessels from the bottom to avoid cracking welded seams and causing leaks. Do not try to reuse culture vessels after freezing, since Nunc cannot control freezing and handling conditions and thus cannot guarantee the integrity of the welded seams. This method must be adjusted by each investigator.

References:

(1) J. H. Hanks and R. E. Wallach (1949) Proc.Soc.Exp.Biol. Med. 71: 196

(2) R. Dulbecco and M. Vogt (1954) J. Exp.Med. 99: 167

General References:

W. B. Jakoby and I. H. Pastand (1979) Methods in Enzymology Volume LVIII: Celle Culture. Academic Press, Inc. NY.

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