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Cold Trypsinization Protocol for Cells Cultured in Serum-Free Media

Introduction

Cells cultured in serum-free media are generally more sensitive to trypsinization than those cultured in serum-supplemented media. Cell viability can drop sharply and the cell growth rate can decline dramatically if trypsinization is not carefully performed. Ideally, trypsinization should be performed on subconfluent rather than confluent monolayers. The following protocol, based on the cold trypsinization procedure described by McKeehan[1], is highly recommended for those using serum-free media. Because this protocol can be used for small as well as large culture vessels (i.e. roller bottles), volumes are left to the discretion of the end-user.

Protocol

1. Remove medium from culture vessels and rinse monolayer with cold (2℃-8℃) UltraSaline A (12-747) for 2-3 minutes.Repeat.
2. Replace UltraSaline A with just enough cold Trypsin-Versene® (EDTA) Solution (17-161) to cover the monolayer.Tighten cap and keep the culture vessel at room temperature for up to 30 seconds.

   NOTE: Some “hard-to-trypsinize” cell types, such as MDCK and human keratinocytes, may require up to three minutes to detach, while others, such as CRFK, require only a few seconds.

3. Aspirate all Trypsin-Versene® from the culture vessel, tighten cap and let the culture vessel stand at room temperature for approximately 2-5 minutes. Dislodge cells by tapping the vessel on the palm of your hand or against a hard surface.The monolayer is sufficiently trypsinized when it can be seen sliding down from the vessel surface upon tapping. If cells do not come off, let the flask stand at room temperature for an additional minute or two.

   NOTE: “Hard-to-trypsinize” cell types may have to be incubated at 35℃-37℃ for up to five minutes. If c ells still do not come off after five minutes in the incubator, repeat the trypsinization procedure with fresh Trypsin-Versene®.

4. Resuspend the trypsinized monolayer in cold UltraSaline A containing 0.1 mg/ml soybean trypsin inhibitor. Pipette suspension gently to break clumps. Keep suspension cold (2℃-8℃) while counting and replating cells. Centrifugation should be avoided because of potential cell damage, but if required, it should be performed at a low speed.

References

1. Wallace L. McKeehan. The effect of temperature during trypsin treatment on viability and multiplication potential of single normal human and chicken fibroblasts. Cell Biology International Reports. Vol. 1 (4):335-343. 1977.

Product Use Statement

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