PRG-2 dPBS-EDTA Solution

Cat# NB-11-0071

Introduction

Cell membranes are materially and cumulatively damaged whenever cells are exposed to serine proteases, physically manipulated, centrifuged, and/or frozen. Use of the PRG greatly minimizes damage and stress to cells during passage or freezing of cell cultures.

PRG-1 (EDTA-dPBS Solution) prepares the cells for PRG-2 (containing Trypsin) processing.
The PRG-2 formulation allows a very substantial reduction (<40%) in the amount of Trypsin (BAEE units/ml) required to detach cells compared to typical commercial trypsin solutions. The trypsin in PRG-2 is stoiciometrically inactivated by trypsin inhibitors in the PRG-3 formulation, preventing nonspecific protease damage to cell membranes after detachment.

Appropriate Use

1. Thaw the three PRG reagents, and store +4°C. Expiration date refers to frozen storage.
2. Warm PRG-1 and PRG-2 to 37°C. Keep PRG-3 in ice-water bath for triple-point temperature.
3. Remove and discard the culture medium gently add PRG-1 sufficient to completely cover the cells.
4. Remove and discard the PRG-1 and immediately add an equal volume of PRG-2.
5. Return the culture to the incubator until cells round up but have not detached (0.5 to 2 min).
6. Release the cells by sharply rapping the culture vessel.
7. Immediately add a volume of ice-cold PRG-3 equal to the volume of PRG-2 used.
8. Remove fluid down to the cell pellet, leaving about 50-100µl of fluid covering the cells.
9. Loosen the pellet by flicking the tube sharply with a finger. Avoid bubbles.
10. Count the cells now (if desired) and adjust.15. Incubate 37°C, 5% CO2, 100% humidity. Feed according to the CSC Medium Kit instructions.
11. Resuspend the cells in CSC Complete Medium (warmed to 37°C) and seed the new culture. Use of Attachment Factor (NB-11-0069) to coat the flask is highly recommended pror to resuspension.
12. Incubate 37°C, 5% CO2, 100% humidity. Feed according to the CSC Medium Kit instructions.

Handling and Storage

Store at -20°C. Once opened, shelf life 30 days at +4 - 8°C

CSC media and reagents are made with WFI, all components are cGMP and ISO Compliant, and are classed "Sterile".

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