Passage Reagent Group (PRG plus Attachment Factor)

Cat# NB-11-0077

Introduction

The Passage Reagent System™ (PRS) is a matched set of CSC Certified™ reagents for releasing cells from culture for subculture or freezing, and Attachment Factor, an extracellular matrix (ECM) product to promote cell attachment to tissue culture surfaces and encourage correct polarity and cytoskeletal organization. The PRS contains four parts: PRG-1 (EDTA -dPBS Solution), PRG-2 (Trypsin/EDTA -dPBS Solution), PRG-3 (Trypsin Inhibitor-dPBS Solution) and Attachment Factor. The chelating agent EDTA in PRG-1 prepares for PRG-2, which contains highly purified trypsin. PRG-3 inactivates the protease in PRG-2 and stabilizes the cell membranes. When re-plating, Attachment Factor promotes adherence of cells to the tissue culture surface. CSC media and reagents are Sterile, made with WFI and all components are cGMP and ISO Compliant.

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.

PRG-3 and Attachment Factor are engineered to work together to stabilize the cell membrane and combine to promote spreading and the establishment of correct polarity after subculture. The PRG is qualified for use with all CSC Certified Media: use of PRG and Attachment Factor are absolutely critical for cells to be passaged or frozen in a Serum-Free Medium System.

Appropriate Use:

Directions for use: passaging cells and re-plating with PRS:

1. Thaw the three PRG reagents and Attachment Factor, and store +4°C. Expiration date refers to frozen storage.
2. Warm PRG-1 and PRG-2 and Attachment Factor 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.
11. Resuspend the cells in CSC Complete Medium (warmed to 37°C) and prepare to seed the new culture.
12. Wet the tissue culture surface(s) with warmed Attachment Factor™ (at 37°C) and remove excess.
13. The surface is now activated for use immediately: rinsing or drying are not required or recommended.
14. Inoculate the culture at once according to Medium Kit instructions.
15. Incubate at 37°C, 5% CO2, 100% humidity. Feed according to 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".