

Passage Reagent Group™

Description: The Passage Reagent Group™ (PRG) is a matched set of certified reagents for releasing cells from culture for subculture or freezing. The PRG contains three parts: PRG-1 (EDTA-dPBS Solution), PRG-2 (Trypsin/EDTA-dPBS Solution) and PRG-3 (Trypsin Inhibitor-dPBS Solution). 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.

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.

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 the PRG-2 is stoichiometrically 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 Certified Media: use of PRG and Attachment Factor™ are absolutely critical for cells to be passaged or frozen in a Serum-Free Medium System.

Uses: Cell culture procedures.

Limitations: Do not use past expiration date.
Do not use if product becomes cloudy.
Product may be frozen one time only.
Expiration date refers to frozen storage only.
Thawed product may be used for 30 days when stored at 2-8°C.

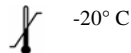
Availability: Kit includes 100ml PRG-1, 100ml PRG-2, and 100ml PRG-3.

Storage: If product is to be used immediately, store at 2-8°C for up to 30 days.
For long-term storage, freeze (one freezing only) at <-20°C.
Expiration date on bottles refers to frozen storage only.

Procedure:

1. Thaw the three PRG reagents, and store at 2-8°C. for up to 30 days. Expiration date on bottle 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, then gently add PRG-1 sufficient to completely cover the cells.

Storage:



-20° C



ScyTek Laboratories, Inc.
205 South 600 West
Logan, UT 84321
U.S.A.



EMERGEOUROPE (31)(0) 70 345-8570
Molsnstraat 15
2513 BH Hague, The Netherlands

Instructions For Use CSC005-IFU

Rev. Date: Aug. 25, 2006

Revision: 2

Page 2 of 2

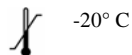
P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 - Fax (435) 755-0015 - www.scytek.com

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 minutes).
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. Transfer this cell suspension to a sterile centrifuge tube. Keep the cells in an ice-water bath.
9. Centrifuge to pellet the cells. A refrigerated centrifuge is strongly recommended.
10. Remove fluid down to the cell pellet, leaving about 50-100µl of fluid covering the cells.
11. Loosen the pellet by flicking the tube sharply with a finger. Avoid bubbles.
12. Count the cells now (if desired) and adjust.
13. Resuspend the cells in Complete Medium (warmed to 37°C) and seed the new culture.
14. Incubate at 37°C, 5% CO₂, 100% humidity. Feed according to the Medium Kit instructions.

Notes:

1. Endotoxin Levels of all PRG reagents are cell culture tested: endotoxin is by chromogenic LAL assay.
2. Sparse plating of cells upon passage (<20% coverage) adversely affects cell growth and is counterproductive.
3. When exposed to PRG some cells will "round up" more quickly than others. This is a function of the ECM (extracellular matrix) produced since the last cell cycle, and the cell cycle stage. It does not necessarily reflect phenotypic variation.
4. ALWAYS pretreat with PRG-1.
5. ALWAYS use an equal volume of PRG-3.
6. ALWAYS keep the temperature of the cell suspension ice-cold and constant. Cells become unhappy when subjected to repeated temperature excursions.
7. ALWAYS avoid bubbles, which present lethal shear stress to your cells. Never vortex for the same reason, squared.
8. Passage Reagent Group™ and Attachment Factor™ are trademark names of Cell Systems Corporation.

Storage:



ScyTek Laboratories, Inc.
205 South 600 West
Logan, UT 84321
U.S.A.



EMERGEOUROPE (31)(0) 70 345-8570
Molsnstraat 15
2513 BH Hague, The Netherlands