



## Passage Reagent System™ (4Z0-840)

**Product Description:** Passage Reagent System™ is a matched set of Cell Systems Certified reagents for releasing cells from culture for subculture or freezing plus Attachment Factor™.

Cell Systems media and reagents are sterile, made with WFI and all components are cGMP and ISO Compliant.

### Components:

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™ each in a 100mL bottle. 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.

**Storage:** Store at -20°C or immediately refrigerate (2 - 8°C). Expiration date refers to frozen storage. Once opened, shelf life is 30 days at +2-8°C.

**Product Use:** 4Z0-840 is for laboratory research only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

**Shipping:** All medium and reagents shipped at ambient temperature.

**Instructions for Use:** Make sure all work surfaces are disinfected. Spray the outside of your gloves prior to work under the hood.

Spray the outside of all tools/instruments (bottles, tubes, racks, etc.) with ethanol solution before bringing them under the hood.

1. Thaw the three PRG reagents, and store at +2°C. Expiration date refers to frozen storage.
2. Warm PRG-1™, PRG-2™, and growth medium to 37°C. Chill the PRG-3™ to 0°C in ice-cold water.
3. Recommended: Prepare a new flask by coating the growth surface with AttachmentFactor™ (cat. #4Z0-210) or other extracellular matrix.
4. Remove growth medium from original flask by pouring into waste receptacle or aspirating.

5. Wash the culture by pipetting 7mL of pre-warmed PRG-1™ into the flask and spreading across the entire growth surface. Then, remove PRG-1™ from flask by pouring into waste receptacle or aspirating.
6. Pipette 7mL of pre-warmed PRG-2™ into the flask and spread across the entire growth surface; immediately move to a microscope for observation.
7. As soon as ~90% of the cells in culture have rounded up, quickly return to the hood and add 7mL of chilled PRG-3™ to the flask, ensuring that the entire growth surface is covered.
8. Pipette the cell suspension up and allow to flow down over the growth surface twice, to wash the surface and to collect any viable cells still adhering to the growth surface. If cells are sticking to the surface, sharply rapping the vessel should release them.
9. Transfer the entire cell suspension volume into a 15mL conical tube.
10. Centrifuge at 900g and +4°C for 10 min.
11. When the centrifuge has stopped, immediately remove the conical tube, disinfect with ethanol solution and move under the hood.
12. Carefully remove medium supernatant from the tube by aspiration or decanting (pouring), ensuring that you do not disturb the cell pellet at the bottom of the tube.
13. Resuspend the cells in the desired volume of warm (37°C) growth medium using a serological pipette. Gently draw the cell suspension up and down in order to mix and break up any cell aggregates. For a typical 1:3 split, resuspend cells in 9mL and transfer 3mL of that in the following step.
14. Transfer the desired volume of cell suspension into the new flask(s) to seed the new culture, and place into incubator (37°C / 5% CO<sub>2</sub> / 95% relative humidity).