

## NPRG-3

#Cat: NB-11-0072 Size: 100ml

### Product Description:

This Reagent is to be used for releasing cells from culture for subculture or freezing cultures. NPRG-3 is intended for use with NPRG-1 and NPRG-2. NPRG-3 inhibits the trypsin activity of NPRG-2, preventing nonspecific protease damage to cell membranes after detachment.

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

### Components:

Quantity 1, 100mL bottle, NB-11-0072, NPRG-3.

### 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:

NB-11-0072 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 NPRG reagents, and store at +2°C. Expiration date refers to frozen storage.
2. Warm NPRG-1, NPRG-2, and growth medium to 37°C. Chill the NPRG-3 to 0°C in ice-cold water.
3. Recommended: Prepare a new flask by coating the growth surface with Attachment Factor (cat. #NB-11-0088) 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 NPRG-1 into the flask and spreading across the entire growth surface. Then, remove NPRG-1 from flask by pouring into waste receptacle or aspirating.
6. Pipette 7mL of pre-warmed NPRG-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 NPRG-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 a new flask(s) to seed the new culture, and place into incubator (37°C / 5% CO<sub>2</sub> / 95% relative humidity).