

**NBP2-32798 Protocol****Product Handling Protocol (NBP2-32798)**

Note: To ensure the highest cell viability, it is strongly recommended that one should thaw the vial and initiate the cell culture as soon as possible upon receipt. If continued storage of the frozen vial upon receipt is necessary, it should be immediately stored in liquid nitrogen but not at -80C. Storage at -80C will lead to significant loss of cell viability. Please read the entire data sheet before thawing. It is recommended that users follow good tissue culture practice. The reporter line is sterile and all work should be performed under sterile conditions.

1. Prepare a sterile 15-ml tube with 9 ml fresh medium without selection agents pre-warmed at 37C.
2. Thaw the frozen cell vial quickly in a 37C water bath, keeping the cap portion out of the water to avoid any possible contamination.
3. Upon thawing, take the vial out of the water and clean it with 70% ethanol to decontaminate.
4. Transfer contents to the 15-ml tube (Step 1) and mix with medium by gentle inversion of tube.
5. Centrifuge at 1,000 RPM for 5 minutes.
6. Remove supernatant and resuspend cells in 10 ml of fresh medium without selection agents. Note: It is important to grow the cells at this stage without selection agents.
7. Transfer cells into a 25-cm<sup>2</sup> tissue culture flask and incubate at 37C in a 95% air-5% CO<sub>2</sub> mixture.
8. After cells settle down (in 1-3 days), remove the medium containing minor floating cells and replace with fresh complete growth medium containing selection agents.
9. Whenever the cells are 70-80% confluent, detach the cells by trypsinization and split into new flasks with fresh complete growth medium.
10. Freeze the reporter cell line at  $3-4 \times 10^6$  cells/ml per cryogenic vial. For optimal cell viability after freezing, freeze cells when they have reached log phase growth (95-98% confluency). Detach cells by trypsinization at 37C for 5 min, and harvest cells by mixing with 3 volumes of fresh medium followed by centrifugation (Step 5). Re-suspend the cell pellet in freeze media (FBS with 10% DMSO). Add cell suspension to cryogenic vials in 1 ml aliquots. Place cryogenic vials, in a tissue culture approved cryogenic vial container, in -80C Freezer for 24-48 hours. After 24-48 hours, move the vials into liquid nitrogen storage.