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NBP2-26261 Protocol

Product Handling Protocol (NBP2-26261)

Please read the entire data sheet before thawing. It is recommended that users follow good tissue culture practice. The RAW cell 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 RAW cell line 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 pellet in 10 ml of fresh medium without selection agents. It is important to grow the RAW cell line at this stage without any selection agents.

7. Transfer the RAW line into a 25-cm2 tissue culture flask and incubate at 37C in a 95% air-5% CO2 mixture.

8. After cells settle down (in 1-3 days), remove the medium and replace with fresh complete growth medium containing selection agents.

9. At 70-80% confluency, detach the cells by trypsinization and split into new flasks with fresh complete growth medium.

10. Freeze the RAW cell line at 3~4 x 10^6 cells/ml per cryogenic vial. For optimal viability after freezing, freeze cells when they have reached log phase growth (95-98% confluency). Detach by trypsinization at 37C for 5 min, and harvest by mixing with 3 volumes of fresh medium followed by centrifugation (Step 5). Resuspend the pellet in freeze media (FBS with 10% DMSO). Add 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.