THAWING PROTOCOL

ThawReady™ by ATCC

ThawReady™ Cell Thawing Protocol

BEFORE THAWING, PREPARE THE FOLLOWING:

- Labeled 15 mL tubes (1 per vial to be thawed)
- Complete medium, pre-warmed
- Just prior to thawing, aliquot 9 mL of warmed complete culture medium to each 15 mL tube

THAWING:

1. Retrieve the desired number of frozen vials from the appropriate LN2 freezer.
2. Quickly transport and place the vials in a floating mat in a 37°C water bath, immersing the vials up to the level of the cap. Thaw for approximately 3 min.
3. Remove all vials from the water bath at the same time and dry with a laboratory wipe. Spray vials with 70% ethanol or equivalent, while gently inverting the vials 2-3 times to mix the contents. Dry vials again with a laboratory wipe. All operations after this point should be carried out under aseptic conditions.
4. Transfer the vials to a biosafety cabinet and aseptically loosen the vial caps. Using P1000 tips, gently and slowly, transfer the cell contents, to the 15 mL sterile centrifuge tube(s) containing 9.0 mL complete growth medium. After transferring all cells, tighten the centrifuge tube caps and mix the cell suspension by gently inverting the centrifuge tube 2-3 times.
5. Centrifuge at 150 to 300 x g for 8 to 12 minutes at room temperature.
6. Aseptically loosen the caps of all centrifuge tubes, discard the supernatant by vacuum, taking care to not disturb the cell pellet.
7. Add 10 mL of pre-warmed complete growth media to each of the tubes. Gently resuspend cell pellet with a 10 mL disposable pipette by pipetting up and down for a few times.
8. Immediately remove an appropriate amount of cell suspension and transfer to a cell counting tube.
9. Transfer tube to your cell counting instrument. Count the cells.
10. Plate cells as appropriate for your assay.

10801 University Boulevard
Manassas, Virginia 20110-2209

703.365.2700 703.365.2701 sales@atcc.org www.atcc.org

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