REF: P20121

CELL CULTURE INSTRUCTIONS

A. Complete Growth medium

- RPMI 1640
- 10% FBS
- 10 μg/ml Puromycin

B. Set up culture after receiving

1. Decontaminate the external surfaces of medium and medium supplements with 70% ethanol.

2. Prepare coated flask (T-75 flask is recommended). Add 9 ml of RPMI 1640 and then add 1ml of FBS (without selection antibiotic). Leave the flask in incubator minimum one hour at 37ºC incubator.

3. Place the vial in a 37ºC water bath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the water bath immediately, wipe it dry, and rinse the vial with 70% ethanol. Remove the cap, being careful not to touch the interior threads with fingers.

4. Dispense the contents of the vial using 1 ml eppendorf pipette and gently resuspend the contents of the vial in T75 flask containing pre-warmed complete growth media.

5. Place the flask in the incubator.

6. For best results, do not disturb the culture for 16 hours after the culture has been initiated. Change the growth medium (including the selection antibiotic) the next day to remove the DMSO and unattached cells, then every other day thereafter.

C. Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.

2. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 0% confluent.

3. Subculture the cells when they are over 90% confluent.

4. Incubate cells with 1 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope). Add 1ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.

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