Serum Inactivation

Take out the frozen serum bottle from -80°C freezer, let it thaw overnight (4°C) or until it is thawed completely. Warm to room temperature for 15-30 mins, before starting the inactivation. Put the serum bottle in a water bath at 56°C for 30 mins, with intermittent shaking. Take the bottle out of the water bath, and let it cool down before putting it back in the refrigerator.

Growth Media Preparation:

| RPMI media (7% Serum) | DMEM media (5% Serum) |
|---|---|
| 1 liter media | 1 liter media |
| 5 ml of Penicillin/Streptomycin (Gibco) | 5 ml of Penicillin/Streptomycin (Gibco) |
| 10 ml of 100X Glutamine (Gibco) | 10 ml of 100X Glutamine (Gibco) |
| 70 ml of heat inactivated serum | 50 ml of heat inactivated serum |

Freezing Media Preparation:

Add to 100ml of RPMI without supplements.

12 g glucose

40 ml DMSO

Filter and store in a bottle covered with aluminum foil (light sensitive).