SHSY5Y Freezing and Thawing Protocol

Freezing:

Equipment:
- Basic Culture Medium
- DMSO
- Centrifuge tube
- Freezing veils

Prepare Freezing Medium (18 ml):
- 17,1 ml Basic Culture Medium
- 900 µl DMSO

Medium should contain 5% (v/v) DMSO

Split cells and count. One freezing veil should contain somewhere around 2-6*10^6 cells. Calculate the number of cells and quantity of desired veils.

- Precool veils and medium to 4°C
- Transfer required amount of cells to a centrifuge tube
- Spin down for 2 minutes at 1.000 x g and 4°C.
- Discard supernatant
- Resuspend cells in 1ml Medium per desired veil
- Transfer 1 ml resuspend cells in each veil
- Keep veils on 4°C for 10 minutes, then at -20°C for further 10 minutes
- Keep cells at -80°C for 24 hours
- Transfer for final storage to liquid nitrogen

Thawing

Equipment:
- Basic Culture Medium
- Veil of frozen SHSY5Y
- Centrifuge tube
- 75 cm^2 culture flask

- Take veil from liquid nitrogen and keep it on ice for 10 minutes
- Rapidly thaw in 37°C water bath until defrosted
- Transfer to 9 ml room temperature Basic Culture Medium
- Invert gently
- Centrifuge for 2 minutes at 1.000 x g
- Discard supernatant
- Resuspend in 10 ml Basic Culture Medium
- Transfer to 75cm^2 flask
- Grow the cells in the Basic Culture Medium at 5% CO2 and 37°C.
- Change medium every 2-3 days for maintaining SHSY5Y cells in culture