Tranferrin Uptake Assay (HeLa cells)

The transferrin receptor (TfR) is a transmembrane protein that mediates cellular uptake of iron. The serum concentration of the soluble TfR (sTfR) is altered in several diseases and used for diagnostic purposes.

Protocol: (Kindly provided by Pablo Lujan from Kohn Group).

1. Seed 1 x 10 cm ø dish with HeLa cells for confluency 24 h after.
2. Rinse cells with pre-warmed serum-free media (SFM) and incubate cells with 7mL SFM 30 min at 37^{o}C, 5% CO_2.
3. Trypsinise cells (1 mL trypsin) during 3 min at 37^{0}C, 5% CO_2.
4. Collect cells with 3mL of SFM and separate them in 8 eppendorf tubes.
5. Centrifuge at 1000 rpm, 3 min, 4^{0}C and resuspend cells in 100µL ice-cold Transferrin Alexa Fluor 633 Conjugate (ThermoFisher, T23362) at a concentration of 50µg/mL in SFM.
6. Incubate on ice 10 min.
7. Incubate at 37^{0}C in different times in duplicate: 0, 2, 5 and 10 min.
8. Centrifuge at 1000 rpm, 3 min, 4^{0}C and resuspend cells in 200µL ice-cold SFM.
9. Centrifuge at 1000 rpm, 3 min, 4^{0}C and resuspend cells in 200µL ice-cold PBS.
10. Centrifuge at 1000 rpm, 3 min, 4^{0}C and resuspend cells in 200µL ice-cold acidic buffer (0.1 M Glycine, 150 mM NaCl, pH 3).
11. Centrifuge at 1000 rpm, 3 min, 4^{0}C and resuspend cells in 200µL ice-cold acidic buffer.
12. **Note:** perform steps 10 and 11 as fast as possible and with no breaks in between. Acidic buffer will kill around 50% of the cells. This percentage increases with time.
13. Centrifuge at 1000 rpm, 3 min, 4^{0}C and resuspend cells in 200µL ice-cold PBS.
14. Centrifuge at 1000 rpm, 3 min, 4^{0}C and resuspend cells in 250µL ice-cold PBS + 100ng/mL DAPI.

15. Filter them with 70µm cell strainer and load them in the proper cytometry tube. Keep it on ice and measure transferrin uptake as soon as possible.

References

Shedding of the Transferrin Receptor Is Mediated Constitutively by an Integral Membrane Metalloprotease Sensitive to Tumor Necrosis Factor Protease Inhibitor-2*. Kaup, M., Dassler, K., Weise, C., and Fuchs, H. (2002) J. Biol. Chem. **277**, 38494-38502.