

## Transferrin 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  $\emptyset$  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<sup>0</sup>C, 5% CO<sub>2</sub>.
3. Trypsinise cells (1 mL trypsin) during 3 min at 37<sup>0</sup>C, 5% CO<sub>2</sub>.
4. Collect cells with 3mL of SFM and separate them in 8 eppendorf tubes.
5. Centrifuge at 1000 rpm, 3 min, 4<sup>0</sup>C and resuspend cells in 100 $\mu$ L ice-cold Transferrin Alexa Fluor 633 Conjugate (ThermoFisher, T23362) at a concentration of 50 $\mu$ g/mL in SFM.
6. Incubate on ice 10 min.
7. Incubate at 37<sup>0</sup>C in different times in duplicate: 0, 2, 5 and 10 min.
8. Centrifuge at 1000 rpm, 3 min, 4<sup>0</sup>C and resuspend cells in 200 $\mu$ L ice-cold SFM.
9. Centrifuge at 1000 rpm, 3 min, 4<sup>0</sup>C and resuspend cells in 200 $\mu$ L ice-cold PBS.
10. Centrifuge at 1000 rpm, 3 min, 4<sup>0</sup>C and resuspend cells in 200 $\mu$ L ice-cold acidic buffer (0.1 M Glycine, 150 mM NaCl, pH 3).
11. Centrifuge at 1000 rpm, 3 min, 4<sup>0</sup>C and resuspend cells in 200 $\mu$ L 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<sup>0</sup>C and resuspend cells in 200 $\mu$ L ice-cold PBS.
14. Centrifuge at 1000 rpm, 3 min, 4<sup>0</sup>C and resuspend cells in 250 $\mu$ L 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.