Endocytotic Uptake Assay for BSF (dextran and ConA) adapted from Morriswood *et al* 2015

Prepare day before (**time = 0h**) : per cell condition :

2.5ml serum-free IMDM at 4°C

1ml 4% PFA-0.1% glutaraldehyde in vPBS (from 32% PFA stock kept at -20°C and 25% glut stock kept at 4°C - open under hood!)

Prepare on the day (**time = 14-18h**): ice box with per cell condition:

1.5ml vPBS and 2.5ml serum-free IMDM

Dextran TMR 10,000 5mg/ml (from 50mg/ml stock)

ConA FITC 10-100µg/ml (from stock 2.5mg/ml)

1ml 4% PFA-0.1% glutaraldehyde in vPBS

1. **Time 0h** = calculate density of cells BSF Tb427 SmOx 10TY p2T7\_FPC6 to 1x105cells/ml, 4x15ml in 75ml flasks.
2. Induce two flasks with 1µg/ml tetracycline (+Tet), keep two flasks non-induced (-Tet).
3. **Time 14-18h =** measure the cell density in control (-􏰃Tet) and induced (􏰄+Tet) cultures, and take 4 samples (2 from each population) of 2x106 cells.
4. **Pellet** the cells by centrifugation (800 x *g* for 10 min at 4°C), then resuspend in 1 ml of ice-cold serum-free IMDM, and transfer to Eppendorf tubes.
5. Pellet by centrifugation (800 x *g* for 5 min at 4°C).
6. Resuspend in 100µ􏰁l of ice-cold serum-free IMDM, and chilled (10 min on ice).
7. **Dextran** (final concentration, 5 mg/ml) and/or **ConA** (final concentration, 10-100 µg/ml) – add 10ul of Dex/Con A to give final concentration and mixed by flicking.
8. The cells are then **incubated** to allow FP loading (15 min on ice).
9. Half the samples are then fixed (time [t] =􏰅 0 min). The other half (t = 30 min) are shifted to higher temperature and incubated to allow uptake (37°C for 30 min).
10. For both sets of samples (t = 0 and 30 min), **uptake is stopped** by the addition of 1 ml of ice-cold serum-free IMDM, and the cells are pelleted (800g x 5mins 4°C) and then resuspended and incubated in 1ml 4% paraformaldehyde – 0.1% glutaraldehyde in ice-cold vPBS (20 min on ice) for **fixation** of the cells.

Mounting on slides

1. The cells are centrifuged at 800g x 5mins at 4°C, resuspended in 50µl vPBS at 4°C, and spread 50µl over a slide well, coated in poly-L-lysine.
2. Cells are left to adhere for 20mins at RT, then dried for 10mins.
3. Wash twice for 10mins with 100mM **glycine**.
4. Permeabilize the cells with **TX-100 (Triton)** in PBS 0.1% for 10mins.
5. Wash twice for 5mins with 100mM glycine.
6. Wash three times for 3-5 mins in PBS.
7. Add 25µl **DAPI** 2mins at RT.
8. Wash three times for 5 mins in PBS.
9. Add a drop of **Slow-fade** to each well (80ul per 10 wells) ; place a coverslip on slide and seal with nail varnish
10. The cells are then imaged directly. The same acquisition settings and exposure times are used for -Tet and +Tet samples.