Cellular DELFIA Assay Protocol

Materials:

- 96 well, white, clear bottom, tissue culture treated plates (Wallac Isoplate TC # 1450-516 or PerkinElmer ViewPlate # 6005181)
- Formaldehyde
- Triton x 100
- PBS
- HEPES
- Bovine Serum Albumin
- DELFIA Assay Buffer # 1244-111
- Primary antibody (mouse or rabbit)
- DELFIA Europium-labeled secondary antibody (mouse # AD0124 or rabbit # AD0105)
- DELFIA Wash Concentrate # 1244-114
- DELFIA Enhancement Solution # 1244-105

Day 1:

- plate cells (density depending on cell line; 20 000/well in 96 well plate for CHO-K1 cells)
- incubate at RT for at least 60 min before placing the plates in the 37ºC CO_2-incubator

Day 2:

- change to serum-free medium for overnight cell starving

Day 3:

- treatment of cells (compounds in culture medium or in PBS 20 mM HEPES)
- fixation (4% formaldehyde & 0.1% Triton in PBS)
- block (0.1% BSA in PBS)
- primary antibody 0.3 – 1µg/mL in DELFIA Assay Buffer at least 2 h

- wash (DELFIA Wash Concentrate, automated cell washer or using multichannel pipette)
- secondary antibody 0.3µg/mL – 1µg/mL in DELFIA Assay Buffer at least 1 h
- wash (DELFIA Wash Concentrate, automated cell washer or using multichannel pipette)
- 200µL Enhancement Solution + 5 min shake for Eu and Sm
- Measure Europium time-resolved fluorescence