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Measurement of heat production (thermogenesis) in cells using ERthermAC dye_microplate method

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MATERIALS TEXT

IBioTracker ERthermAC Temperature-Sensitive Live Cell Dye (SCT057, Merck) (https://www.merckmillipore.com/TW/zh/product/BioTracker-ERthermAC-Temperature-Sensitive-Live-Cell-Dye,MM_NF-SCT057?ReferrerURL=https%3A%2F%2Fl.facebook.com%2F&fbclid=IwAR1xNHOYdLL4iTg8IRPS0fHiwa88ZmXFFCu2vYnZTVg4ivkjz3_YKd1AFb8)

IPrepare stock solution of ERthermAC dye (1 mM in DMSO)

IBlack 96-well plate with clear bottom

BEFORE STARTING

IDifferentiate brown/white adipocytes in a black 96-well plate with clear bottom

IPrepare staining medium: 250 nM ERthermAC dye in DMEM-H serum-free medium

1 Wash differentiated adipocytes with PBS twice

2 Add 100 µL of staining solution per well of a 96-well plate

3 Incubate 37oC for 30~45 min

4 Wash with PBS twice

5 Add 90 ul of DMEM-H serum-free and phenol red-free medium each well

6 Equilibrate plate at 25 oC for 15 min

7 Measure 2-3 points of basal red fluorescence (ex/em, 543/590) in a microplate reader

8 Add 10 uL of medium or 10 mM CL-316,243 (CL)/forskolin (Fsk) each well (10x dilution, final 1 µM)

9 Measure the red fluorescence (ex/em, 543/590) every 5 min up to 1.5 ~ 2 hr

10 Calculate the decrease of fluorescence over time after drug treatment

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