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Cell culture, transfection, and imaging of K562 cells V.1

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We use this protocol and it's working

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Abstract

This protocol details the general preparation of K562 cells for imaging.

Cell culture

2d 17h 30m

1 Culture K562 cells at 37 °C and 5% CO2 in RPMI containing 10% FBS, [M] 1 millimolar (mM) sodium pyruvate, [M] 100 Mass Percent penicillin, [M] 100 Mass Percent streptomycin, [M] 2 millimolar (mM) L-glutamine, [M] 1 Mass Percent non-essential amino acids, (all from Gibco) and [M] 2.5 Mass Percent plasmocin (InvivoGen).

Note

K562 cells are maintained between 1x10^5 and 1x10^6 cells/mL, passaging when reaching confluency (1x10^6 cells/ml) or otherwise renewing media every 2-3 days.

Transfection and imaging

2d 17h 30m

2 For imaging experiments, seed the cells on fibronectin (Sigma Aldrich) 35mm imaging dishes (MatTek) at a concentration of 2×10^5 cells per dish in RPMI without antibiotics. Allow cells to attach Overnight at 37 °C and 5% CO2.

16h

3 After cells attach, replace media with RPMI supplemented with hemin (Sigma Aldrich) dissolved in DMSO to a final concentration of [M] 30 micromolar (µM). Transfect transiently with plasmids by adding FuGene 4K (Promega) and incubate Overnight. After the overnight incubation, replace the media with new media containing the same factors of hemin and (where applicable) transfection reagent and plasmids, and incubate Overnight.

2d

4 Following the 2 days of transfection/hemin treatment, prepare the cells for imaging. Where applicable, add Halo ligand and incubate for 01:30:00 at 37 °C, 5% CO2. Replace with new RPMI media (supplemented at [M] 30 micromolar (µM) hemin if differentiating cells) prior to imaging.

If using TMRE (Cayman Chemical, TMRE Mitochondrial Membrane Potential Assay Kit), add prior to imaging at a final concentration of [M] 200 nanomolar (nM) TMRE.

1h 30m

5 Perform spinning-disk confocal microscopy using an Andor Dragonfly system equipped with a plan apochromat objective (63×, 1.4 NA, oil) and a Zyla scientific CMOS camera. Cells are imaged at 37 °C and 5% CO2.

6 Identify the cells to be imaged by scanning the dish.