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

Chase Amos[1], Pietro De Camilli[1]

[1]1Departments of Neuroscience and of Cell Biology, Howard Hughes Medical Institute, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, Connecticut 06510, USA 2Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

Chase Amos

VERSION 1

DEC 13, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.e6nvwdk4dlmk/v1

Protocol Citation: Chase Amos, Pietro De Camilli 2023. Cell culture, transfection, and imaging of K562 cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwdk4dlmk/v1 Version created by Chase Amos

Protocol status: Working

We use this protocol and it's working

ABSTRACT

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

Created: Dec 13, 2023

Last Modified: Dec 13, 2023

PROTOCOL integer ID: 92286

Keywords: ASAPCRN

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 (MatTe 16h at a concentration of 2x10^5 cells per dish in RPMI without antibiotics. Allow cells to attach Overnight at 37 °C and 5% CO2.

3 After cells attach, replace media with RPMI supplemented with hemin (Sigma Aldrich) dissolved in DM 2d 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 .

4 Following the 2 days of transfection/hemin treatment, prepare the cells for imaging. Where applic 1h 30m 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.

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.