

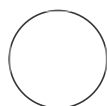


VERSION 1  
DEC 13, 2023

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

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### ABSTRACT

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

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#### DOI:

[dx.doi.org/10.17504/protocols.io.e6nvwdk4dlmk/v1](https://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.

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<https://dx.doi.org/10.17504/protocols.io.e6nvwdk4dlmk/v1>

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**Protocol status:** Working

We use this protocol and it's working

**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  $37^{\circ}\text{C}$  and 5% CO<sub>2</sub> in RPMI containing 10% FBS, 1 millimolar (mM) sodium pyruvate, 100 Mass Percent penicillin, 100 Mass Percent streptomycin, 2 millimolar (mM) L-glutamine, 1 Mass Percent non-essential amino acids, (all from Gibco) and 2.5 Mass Percent plasmocin (InvivoGen).

### Note

K562 cells are maintained between  $1 \times 10^5$  and  $1 \times 10^6$  cells/mL, passaging when reaching confluency ( $1 \times 10^6$  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), 16h at a concentration of  $2 \times 10^5$  cells per dish in RPMI without antibiotics. Allow cells to attach Overnight at  $37^{\circ}\text{C}$  and 5% CO<sub>2</sub>.
- 3 After cells attach, replace media with RPMI supplemented with hemin (Sigma Aldrich) dissolved in DMSO, 2d to a final concentration of 30 micromolar ( $\mu\text{M}$ ). 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 applicable, 1h 30m add Halo ligand and incubate for 01:30:00 at  $37^{\circ}\text{C}$ , 5% CO<sub>2</sub>. Replace with new RPMI media (supplemented at 30 micromolar ( $\mu\text{M}$ ) 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% CO<sub>2</sub>.
- 6 Identify the cells to be imaged by scanning the dish.