Jan 26, 2026

K562 culture and characterization

DOI
https://dx.doi.org/10.17504/protocols.io.5qpvo1krbg4o/v1

Lexi Bounds[1], Alejandro Barrera[1], Maria ter Weele[1], Siyan Liu[1], Euphy Wu[2], Shengyu Li[1], Revathy Venukuttan[1], Ruhi Rai[1], Wancen Mu[2], Nahid Iglesias[1], Paola Giusti-Rodriguez[3], Timothy Reddy[1], Yun Li[2], Reluca Gordan[4], Andrew Allen[1], Michael Love[2], Patrick Sullivan[2], Greg Crawford[1], Charles Gersbach[1]

[1]Duke University; [2]University of North Carolina; [3]University of Florida; [4]University of Massachusetts

Gersbach Lab

Andrea R Daniel
Duke University

DOI: https://dx.doi.org/10.17504/protocols.io.5qpvo1krbg4o/v1

Protocol Citation: Lexi Bounds, Alejandro Barrera, Maria ter Weele, Siyan Liu, Euphy Wu, Shengyu Li, Revathy Venukuttan, Ruhi Rai, Wancen Mu, Nahid Iglesias, Paola Giusti-Rodriguez, Timothy Reddy, Yun Li, Reluca Gordan, Andrew Allen, Michael Love, Patrick Sullivan, Greg Crawford, Charles Gersbach 2026. K562 culture and characterization. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvo1krbg4o/v1

Protocol status: Working
We use this protocol and it's working

Created: January 22, 2026

Last Modified: January 26, 2026

Protocol Integer ID: 239164

Keywords: K562 cells, dCas9-KRAB, k562 cell culture, methods for k562 cell culture, k562 culture

Funders Acknowledgements:
NIH
Grant ID: HG012053

Disclaimer

This protocol was adapted from the work of Lexi Bounds in the Gersbach lab at Duke University.

Abstract

This protocol describes methods for K562 cell culture and characterization.

Materials

dCas9-KRAB (Addgene #83890)
hygromycin B (ThermoFisher #10687010)
Blasticidin S HCl (ThermoFisher #A1113903)
Invitrogen™ SuperScript™ VILO™ cDNA Synthesis Kit (ThermoFisher #11754050)
PerfeCTa SYBR Green FastMix (Quantbio #95072)

K562 culture

1 K562 cells are obtained from the American Tissue Collection Center (ATCC) via the Duke University Cancer Center Facilities.

2 K562 cells are cultured in RPMI1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin.

3 The polyclonal dCas9-KRAB cell line are generated as previously described (ref 1). K562 cells are transduced with dCas9-KRAB (Addgene #83890) lentivirus with polybrene at a concentration of 8 ug/mL. At two days post-transduction, cells are selected for 10 days with 600 ug/mL hygromycin B (ThermoFisher #10687010). Following selection, polyclonal cells are stained to confirm expression of dCas9-KRAB protein.

K562 Characterization

4 dCas9-KRAB K562 cells are transduced with an individual gRNA targeting the HS2 enhancer region (ref 2) or transduced with a nontargeting gRNA (N=3 each) via spinfection. 25,000 cells are resuspended in 0.5 mL of media containing 25 uL of lentivirus and 8 ug/mL polybrene, then centrifuged for 30 minutes at 25C

5 The volume is transferred into one well of a 24-well plate.

6 24 hours post-transduction, the cells are centrifuged for 5 minutes at 300g, resuspended in fresh media, and plated into a 6-well plate.

7 Cells are then selected with 2.5ug/mL Blasticidin S HCl (ThermoFisher #A1113903) for 3 days.

8 The cells are harvested and mRNA was purified using the Qiagen RNeasy kit according to the manufacturer's protocol.

9 250 ng mRNA is used as input for cDNA amplification using the Invitrogen™ SuperScript ™ VILO™ cDNA Synthesis Kit (ThermoFisher #11754050).

10 For RT-qPCR, each reaction contains 2 uL cDNA, 6 uL H20, 1 uL 10 uM FWD primer, 1 uL 10uM RVS primer, and 10 uL PerfeCTa SYBR Green FastMix (Quantbio #95072).

11 Delta delta Ct analysis is performed in Microsoft Excel. Graphpad Prism is utilized to conduct one-way ANOVA tests followed by Tukey's HSD for post-hoc testing.

Protocol references

1. Klann, T. S. *et al.* Genome-wide annotation of gene regulatory elements linked to cell fitness. 2021.03.08.434470 Preprint at https://doi.org/10.1101/2021.03.08.434470 (2021).

2. Thakore, P. I. *et al.* Highly specific epigenome editing by CRISPR-Cas9 repressors for silencing of distal regulatory elements. *Nat. Methods* **12**, 1143–1149 (2015).