TECHNICAL MANUAL

Edit-R™ Cas9 and CRISPRmod CRISPRa dCas9-VPR stable cell lines

Cat.#	
HD Cas9-001	HD Cas9-014
HD Cas9-002	HD dCas9-VPR-002
HD Cas9-004	HD dCas9-VPR-005
HD Cas9-005	HD dCas9-VPR-011
HD Cas9-007	HD dCas9-VPR-012
HD Cas9-010	HD dCas9-VPR-014
HD Cas9-011	
HD Cas9-012	

Production

The Cas9 and dCas9-VPR Stable Cell Lines were generated by transduction using Edit-R Cas9 nuclease lentiviral particles, or CRISPRmod CRISPRa dCas9-VPR lentiviral particles followed by Blasticidin selection for 14 days (see concentration in Appendix, Table 4). The Cas9 and dCas9-VPR stable adherent cell lines are supplied as a single vial containing > 1×10^6 viable cells in 1 mL of freezing medium (includes 50% Fetal Bovine Serum (FBS) and 5% DMSO). While the Cas9 and dCas9-VPR suspension lines, Jurkat and K-562, are supplied as a single vial containing > 1 × 106 viable cells in 1 mL of freezing medium (includes 50% FBS and 10%DMSO).

Shipping and storage

The Cas9 and dCas9-VPR stable cell lines are shipped on dry ice. While precautions have been taken to prevent CO_2 from entering the vial during shipment, it is suggested that upon receipt the cells be stored for two days or more in liquid nitrogen to allow any CO_2 to dissipate. When removing the vial from liquid nitrogen storage, leave at room temperature for approximately 30 seconds or longer to allow the liquid nitrogen to dissipate from the vial. **Note: Always wear protective eyewear and gloves when handling vials stored in liquid nitrogen.**

Quality Control

The Cas9 and dCas9-VPR stable cell lines have been tested for viability and verified to contain no mycoplasma contamination. Cas9 and dCas9-VPR activity was confirmed by functional gene editing assay and RT-qPCR assay, respectively, after transient transfection with synthetic Positive and Negative Control guide RNAs. The quality control measures can be found in full in the batch specific Certificate of Analysis.

Starting cells from frozen cell stock (thaw quickly)

1. Remove the Cas9 and/or dCas9-VPR stable cell line from liquid nitrogen and place in a 37 °C water bath for 2 minutes until nearly (~ 80%) thawed.
2. Remove the cells from the vial and add slowly into a 15 mL conical tube containing 9 mL pre-warmed cell culture medium (see appendix, Table 2).
3. Centrifuge for 4 minutes at 300 × g to pellet cells and remove the supernatant.
4. Add 2 mL of the appropriate cell culture medium and transfer cells to a T25 flask containing 4 mL of pre-warmed cell culture medium.
5. Place the cells in a humidified 37 °C incubator with 5% CO_2.
6. For adherent cell lines, gently replace medium after 24 hours with 5-10 mL appropriate cell culture medium and continue to culture at 37 °C with 5% CO_2. For suspension cell lines (K-562 and Jurkat), allow cells to recover for a few days until approaching 1 x10^6 cells/mL to perform cell count and viability check. (The culture should not exceed 1 x 10^6 cells/mL.)

Subculturing cells

We recommend that you passage cells at least once before using them in your desired application.

The cell culture guidelines and volumes below describe the subculturing protocol we use to passage cells in a standard T75 cell culture flask. When using different flasks or plates, culture volumes should be increased or decreased as per manufacturer's guidelines.

Protocol for adherent Cas9 and dCas9-VPR Stable Cell Lines

Cell lines: A549, HCT-116, RKO, NIH/3T3, HAP1, U2OS, and MDA-MB-231.

Cell lines are typically passaged when 70-90% confluent. More specific details on each cell line can be found in Appendix, Table 2.

1. Carefully aspirate the growth medium from the cells. This is best done by tilting the flask or plate and removing the medium without touching the cell surface.
2. Gently wash cells with 7.5 mL PBS to remove the remaining media.
3. Trypsinize the cells with 3 mL Trypsin-EDTA solution (GIBCO, Cat. #25300096). Place the flask in the 37 °C incubator for approximately 2 minutes or until the cells release from the flask.
4. Add 15-30 mL of the appropriate cell culture medium described in the Appendix (Table 2) to resuspend the detached cells and inactivate the trypsin.

5. Pipette cells up and down ~ 5 times with a 10 mL pipette to obtain a single cell suspension, while avoiding frothing of medium.
6. Plate cells according to split ratios recommended in Appendix (Table 2) into new sterile flasks or plates containing appropriate cell culture medium. Place the cells in a humidified 37 °C incubator with 5% CO_2.

Protocol for suspension Cas9 and dCas9-VPR Stable Cell Lines

Cell Lines: Jurkat and K-562.

Cell lines are typically maintained at a cell density between 1 x 10^5 and 1x 10^6 viable cells/mL. More specific details on each cell line can be found in Appendix, Table 2.

1. Make sure the cells are evenly distributed in the medium and carefully take a small sample (e.g. 100 µL) of the cells from the cell suspension and determine the total number of viable cells using a cell counter.
2. Calculate the volume of appropriate cell culture medium (See Appendix, Table 2) needed to reach a seeding density of 1 x 10^5 cells/mL using the formula below.

$$Volume\ of\ Fresh\ Medium = \frac{Cell\ Density \times Cell\ Volume}{1 \times 10^5\ cells/mL} - \text{Cell Volume}$$

3. Re-seed desired number of cells into the new sterile flasks or plates containing appropriate cell culture medium. Place the cells at 37 °C with 5% CO_2.

Freezing Cas9 and dCas9-VPR Stable Cell Lines

1. Trypsinize (for adherent cell lines) and centrifuge cells for 5 minutes at 1200 rpm or 300 x g.
2. Resuspend cell pellet in their appropriate freezing medium as described in Appendix, Table 3.
3. Transfer cell suspension to suitable cryovials.
4. Be sure to transfer the cryovials to the freezer within 5 minutes after addition of freezing medium. Place tubes in a suitable freezing container in a -80 °C freezer in order to allow slow cooling.
5. The following day, transfer cryovials to liquid nitrogen.

Technical Tips: We strongly recommend freezing down and using cells at lower passage numbers.

Guide RNA delivery

For synthetic guide RNA delivery, we recommend using the transfection method that is optimal for the background cell line and optimizing the delivery condition using synthetic Positive and Negative Control guide RNAs.

For background cell lines that can be transfected with a lipid-based transfection reagent, we recommend using DharmaFECT transfection reagent (see Table 1 for recommended DharmaFECT reagent).

DharmaFECT Formula	Names of Background Cell Lines
DharmaFECT 1	A549, RKO, NIH/3T3, HAP1
DharmaFECT 4	HCT-116, U2OS, MDA-MB-231

Table 1. Recommended DharmaFECT formula for Cas9 and dCas9-VPR Stable Cell Lines

For background cell lines that prefer electroporation, such as Jurkat and K-562, we would recommend following the electroporation instrument manufacturer's protocol.

Frequently Asked Questions (FAQs)

1. Should I maintain Blasticidin selection pressure while culturing the Cas9 and dCas9-VPR stable cell line?

It depends on the downstream application.

As the Cas9 or dCas9-VPR expressing construct with Blasticidin selection marker was integrated into the cell genome following lentiviral transduction, maintaining Blasticidin pressure is not necessary for a single gene editing or activation experiment.

However, maintaining Blasticidin selection pressure may be desired to prevent potential silencing of Cas9 or dCas9-VPR expression for certain applications such as CRISPR or CRISPRa screening assays. In this case, Blasticidin is removed during transfection and re-applied 2-3 days post-transfection. Please see Table 4 for recommended Blasticidin concentration for each cell line.

2. How do I know if Cas9 or dCas9-VPR is expressed in the Cas9 and dCas9 stable cell line?

During quality control, Cas9 and dCas9-VPR activity was confirmed by functional gene editing and RT-qPCR assay, respectively, after transient transfection with synthetic Positive and Negative Control guide RNAs.

When using the Cas9 stable cell Lline, we recommend using one of our synthetic guide RNA Positive Control kits (including mismatch assay primers) and performing a mismatch assay to assess the editing efficiency and optimize guide RNA delivery condition. Performing mismatch detection assays when using any gene-specific guide RNA to determine editing efficiency for all experiments, even after the delivery conditions have been optimized, is also recommended.

When using the dCas9-VPR stable cell line, we recommend using one of our synthetic CRISPRa positive crRNA controls and performing RT-qPCR to optimize guide RNA delivery condition.

3. My HAP1 Cas9/dCas9-VPR stable cells appeared to be round and lifted off the plates after thawing, are they dead?

The HAP1 cells are adherent, however, they do round up and lift from the flask during S phase of the cell cycle. Performing half media changes after revival can help avoid the removal of actively dividing cells.

4. What happens if I exceed the cell densities recommended?

Exceeding maximum recommended density will affect health and viability of the cells. If cell densities are exceeded, the best practice is to start with a fresh stock.

Appendix

Cell culture medium and cell line maintenance tips

Cell culture medium and cell line maintenance tips can be found in Table 2.

Background cell line	Cat#	Basal medium	Supplements	Subculturing tips
A549	HD Cas9-001	RPMI 1640	10% FBS 1% Pen/Strep	• Recommended split ratio - 1:3 to 1:8 • Maintain cultures between 6 x 10^3 - 6 x 10^4 cells/cm^2 • Cultures should not exceed 7 x 10^4 cells/cm^2
HCT-116	HD Cas9-002 HD dCas9-VPR-002	RPMI 1640	10% FBS 1% Pen/Strep	• Recommended split ratio - 1:3 to 1:8 • Split cultures at 75-90% confluency
Jurkat	HD Cas9-004	RPMI 1640	10% FBS 1% Pen/Strep	• Maintain cultures between 1 x 10^5 and 1 X 10^6 cells/mL
K-562	HD Cas9-005 HD dCas9-VPR-005	IMDM	10% FBS 1% Pen/Strep	• Maintain cultures between 1 x 10^5 and 1 X 10^6 cells/mL
RKO	HD Cas9-007	RPMI 1640	10% FBS 1% Pen/Strep	• Recommended split ratio - 1:3 to 1:12 • Split cultures at 75-90% confluency
NIH/3T3	HD Cas9-010	DMEM, high glucose, GlutaMAX™	10% FBS 1% Pen/Strep	• Seed 3-5 x 10^3 cells/cm^2 • Split cultures at 80% confluence or less • Do not allow the cultures to become confluent
HAP1	HD Cas9-011 HD dCas9-VPR-011	IMDM	10% FBS 1% Pen/Strep	• Recommended split ratio - 1: 10 to 1: 15 • Do not allow cells to be kept at high density, maximum density: 75% confluency
U2OS	HD Cas9-012 HD dCas9-VPR-012	DMEM, high glucose	10% FBS 1% Sodium Pyruvate 1% Pen/Strep	• Recommended split ratio - 1:3 to 1:6 • Split at 75-90% confluency
MDA-MB-231	HD Cas9-014 HD dCas9-VPR-014	DMEM/F-12	10% FBS 1% Pen/Strep	• Recommended split ratio - 1:2 to 1:4 • Split cultures at 75-90% confluency

Table 2. Cas9 and dCas9-VPR stable cell line culture medium

Abbreviation and catalog numbers:

FBS: Fetal Bovine Serum
Pen/Strep: Penicillin/Streptomycin
RPMI 1640: Roswell Park Memorial Institute Medium (Thermo Fisher, Cat. #21875-034)
DMEM/F-12: Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (Thermo Fisher, Cat. #31330-038)
IMDM: Iscove's Modified Dulbecco's Medium (Thermo Fisher, Cat. #21980-032)
DMEM, high glucose: Dulbecco's Modified Eagle Medium, high glucose (Thermo Fisher, Cat. #41965-039)
DMEM, high glucose, GlutaMAX™: Dulbecco's Modified Eagle Medium, high glucose (Thermo Fisher, Cat. #61965-026)

Recommended freezing medium for Cas9 and dCas9-VPR stable cell lines

The recommended freezing medium is composed of the appropriate Basal Media as described in Table 2, 50% FBS, and appropriate percentage of DMSO as described in Table 3.

Freezing medium	Names of background cell lines
45% Basal Medium 50% FBS 5% DMSO	A549, HCT-116, RKO, NIH/3T3, HAP1, U2OS, and MDA-MB-231
40% Basal Medium 50% FBS 10% DMSO	Jurkat and K-562

Table 3. Recommended freezing medium for Cas9 and dCas9-VPR stable cell lines

Recommended Blasticidin selection concentrations for Cas9 and dCas9-VPR stable cell lines

The Cas9 and dCas9-VPR stable cell lines were Blasticidin selected for 14 days during production, see Blasticidin concentration for each cell line in Table 4 should you desire to re-apply the selection pressure.

Blasticidin concentration	Names of background cell lines
5 µg/mL	Jurkat, NIH/3T3
10 µg/mL	A549, HCT-116, K-562, RKO, U2OS, MDA-MB-231
15 µg/mL	HAP1

Table 4. Recommended Blasticidin selection concentrations

For more information

To find the contact information in your country for your technology of interest, please visit us at **horizondiscovery.com/contact-us**

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