CELLECTA

USER MANUAL

CRISPRiTest™ Functional dCas9-Repressor Assay Kit

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Cellecta, Inc.

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1. CRISPRiTest™ Functional dCas9-Repressor Assay Kit

The CRISPRiTest™ Functional dCas9-Repressor Assay Kit allows you to measure a functional dCas9-Repressor (e.g., dCas9-KRAB) in most mammalian cell systems. The choice of kit CRISPRiTest™ or CRISPRiTest™ (EF1L), depends on the cell line to be tested. In iPS cells, where the CMV promoter used in the CRISPRuTest™ Kit is quickly silenced, the CRISPRuTest™ Kit (EF1L) is strongly recommended.

The CRISPR interference (CRISPRi) system is a modification of the original CRISPR system that uses a targeted sgRNA molecule to direct a “catalytically dead” Cas9 (dCas9) fused with a gene repressor, for example, KRAB (i.e., dCas9-KRAB), to a target a gene promoter on genomic DNA. The recruitment of the Cas9-Repressor to the promoter region then blocks the expression of the gene regulated by the promoter. The CRISPRiTest Kit provides reagents to measure the repressor efficiency of any *Streptococcus pyogenes* dCas9 repressor (dCas9-Rep) hybrid protein in cells designed to be used for CRISPRi experiments, using a FACS-based assay. The Kit contains packaged lentiviral constructs expressing dCas9-Rep regulated GFP and a non-regulated RFP protein. The Kit provides enough reagents for 5-10 cell lines.

References and Product Citations for all Cellecta products can be found on the Cellecta website: https://cellecta.com/pages/citations-publications.

Please read the entire user manual before proceeding with your experiment. Also, please note that, when working with pseudoviral particles, you should follow the recommended guidelines for working with Biosafety Level 2 (BSL-2) materials.

Click the ? Download as PDF link located at the bottom of the left menu to download the PDF version of this user manual.

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2. Kit Components

The CRISPRiTest™ comes in two formats:

- CRISPRiTest™ has the CMV promoter:

Component	Volume
CRISPRiTest™ Virus (CiT Viral Mix)	500 µl x 3 vials
Transduction Reagent (1000X)	50 µl

- CRISPRiTest™ (EF1L) has the EF1L promoter:

Component	Volume
CRISPRiTest™ Virus (CiT EF1L Viral Mix)	500 µl x 3 vials
Transduction Reagent (1000X)	50 µl

The CRISPRiTest Kit should be stored at -80°C until ready for use.

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3. Additional Required Materials

Use of the CRISPRiTest assay requires access to a flow cytometer with the following excitation and emission requirements:

> **Excitation:** 488nm and 561nm
> **Emission:** 530/20nm and 590/20nm

Cellecta also offers the following products that may be useful when running this assay:

dCas9-Only Plasmids

Cat.#	Description	Quantity
SVKRABC9B-PS	CRISPRi dCas9-KRAB pRDKCCB-CMV-dCas9-KRAB-2A-Blast (plasmid)	25 µg
SVKRABC9B-VS	CRISPRi dCas9-KRAB pRDKCCB-CMV-dCas9-KRAB-2A-Blast (virus)	1×10^6 TU
SVKRABC9H-PS	CRISPRi dCas9-KRAB pRDKCCB-CMV-dCas9-KRAB-2A-Hygro (plasmid)	25 µg
SVKRABC9H-VS	CRISPRi dCas9-KRAB pRDKCCB-CMV-dCas9-KRAB-2A-Hygro (virus)	1×10^6 TU
SVKRABC9B-PS	CRISPRi dCas9-KRAB pRDKCCBla-CMV-dCas9-KRAB-2A-Blast (plasmid)	25 µg
SVKRABC9B-VS	CRISPRi dCas9-KRAB pRDKCCBla-CMV-dCas9-KRAB-2A-Blast (virus)	1×10^6 TU
SVKRABC9G-PS	CRISPRi dCas9-KRAB pRDKCCG-CMV-dCas9-KRAB-2A-TagGFP2 (plasmid)	25 µg
SVKRABC9G-VS	CRISPRi dCas9-KRAB pRDKCCG-CMV-dCas9-KRAB-2A-TagGFP2 (virus)	1×10^6 TU
SVKRABC9H-PS	CRISPRi dCas9-KRAB pRDKCCH-CMV-dCas9-KRAB-2A-Hygro (plasmid)	25 µg
SVKRABC9H-VS	CRISPRi dCas9-KRAB pRDKCCH-CMV-dCas9-KRAB-2A-Hygro (virus)	1×10^6 TU
SVKRABC9N-PS	CRISPRi dCas9-KRAB pRDKCCN-CMV-dCas9-KRAB-2A-Neo (plasmid)	25 µg
SVKRABC9N-VS	CRISPRi dCas9-KRAB pRDKCCN-CMV-dCas9-KRAB-2A-Neo (virus)	1×10^6 TU
SVKRABC9R-PS	CRISPRi dCas9-KRAB pRDKCCR-CMV-dCas9-KRAB-2A-TagRFP (plasmid)	25 µg
SVKRABC9R-VS	CRISPRi dCas9-KRAB pRDKCCR-CMV-dCas9-KRAB-2A-TagRFP (virus)	1×10^6 TU
SVKRABC9EB-PS	CRISPRi dCas9-KRAB pRDKCEBla-EF1-dCas9-KRAB-2A-Blast (plasmid)	25 µg
SVKRABC9EB-VS	CRISPRi dCas9-KRAB pRDKCEBla-EF1-dCas9-KRAB-2A-Blast (virus)	1×10^6 TU
SVKRABC9ER-PS	CRISPRi dCas9-KRAB pRDKCER-EF1-dCas9-KRAB-2A-TagRFP (plasmid)	25 µg
SVKRABC9ER-VS	CRISPRi dCas9-KRAB pRDKCER-EF1-dCas9-KRAB-2A-TagRFP (virus)	1×10^6 TU
SVKRABC9E2B-PS	CRISPRi dCas9-KRAB pRDKCE2Bla-EFS-dCas9-KRAB-2A-Blast (plasmid)	25 µg
SVKRABC9E2B-VS	CRISPRi dCas9-KRAB pRDKCE2Bla-EFS-dCas9-KRAB-2A-Blast (virus)	1×10^6 TU
SVKRABC9RB-PS	CRISPRi dCas9-KRAB pRDKCRBla-RSV-dCas9-KRAB-2A-Blast (plasmid)	25 µg
SVKRABC9RB-VS	CRISPRi dCas9-KRAB pRDKCRBla-RSV-dCas9-KRAB-2A-Blast (virus)	1×10^6 TU

Cell Line

Parental dCas9-KRAB+ MDA-MB-231 Cells (Cat.# ZMDAMB231-CKRA, 10^6 cells) may be run in parallel with the user's dCas9-repressor (dCas9-Rep) cells as a positive control for the assay.

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Transduction Control Virus

Non-Targeting CRISPR Control pRSG16-U6-sg-UbiC-TagRFP-2A-Puro (virus) may be used to assess transduction efficiency of the target cells.

Cat.#	Description	Quantity
SGCTL-NT-pRSG16-V	Non-Targeting CRISPR control pRSG16-U6-sg-UbiC-TagRFP-2A-Puro Vector (virus)	1×10^7 TU

Non-targeting controls in other Cellecta vectors are available. Please visit our CRISPR Non-Targeting sgRNA Control Construct in Standard Lentiviral Vector web page.

Other than the specific reagents and instruments above, the protocol assumes the user has access to standard materials (e.g., polypropylene tubes, pipette tips), equipment (table top centrifuges, pipettes, scales), and common reagents (e.g., TE buffer, ethanol) and buffers used in a typical life science laboratory.

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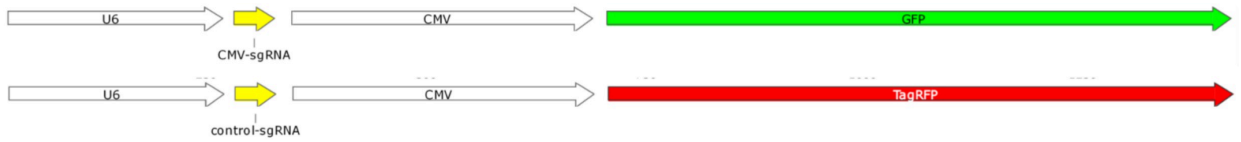
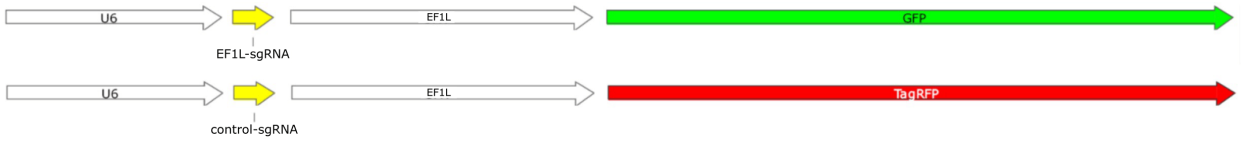
4. Protocol Overview

The CRISPRiTest™ Virus (CiT Viral Mix) contains two premixed lentivectors: (1) a vector expressing GFP from the CMV promoter and a U6-driven sgRNA targeting the CMV-GFP transcription start site and (2) a vector expressing RFP from the CMV promoter and a U6-driven non-targeting sgRNA.



CRISPRiTest CiT Virus Mix



CRISPRiTest CiT EF1L Virus Mix

Transduction of CiT Viral Mix into parental cells will result in a mixed population of transduced cells expressing high levels of RFP and high levels of GFP. Transduction of CiT virus mix into dCas9-KRAB cells will result in a mixed population of transduced cells expressing high levels of RFP and low levels of GFP.

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4.1. Assay Procedure

Parental and dCas9-Rep cells are transduced with the CRISPRiTest™ Virus (CiT Viral Mix). After 4 days, cells are analyzed by flow cytometry to compare the mean RFP and GFP fluorescence levels. The mean GFP and RFP fluorescent values are then used to calculate dCas9-Rep activity in dCas9-Repressor cells.

The assay was optimized using MDA-MB-231 cells. Parental and dCas9-KRAB MDA-MB-231 cells are available from Cellecta to use in the assay as a positive control (see Additional Required Materials). Some optimization may be needed based on the growth characteristics of your target cells.

Start the experiment with actively growing cells. Maintain cells in log phase throughout the entire experiment.

Day 0

1. Quickly thaw the CiT Viral Mix in a water bath at 37°C. Transfer the thawed particles to a laminar flow hood, gently mix by rotation, inversion, or gentle vortexing, and keep on ice. Unused reagent can be aliquoted, refrozen at -70°C, and used again for subsequent experiments.

2. Suspend both parental and dCas9-Rep cells in growth medium supplemented with 1X CRISPRTest Transduction

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Reagent, at a density of ca. 100,000 cells/ml.

3. For each cell line, aliquot 1ml of cell suspension/well in 1 well of a 12-well plate.

4. Add 40µl of CiT Viral Mix to each well, mix and return cells to incubator.

> **Note:** For most cell lines, 40µl of CiT Viral Mix would suffice to obtain at least 70% RFP+ cells (the recommended minimum % of transduced cells for optimal assay sensitivity). For hard to transduce cells, more virus (and/or spinoculation) might be needed. When in doubt, it is recommended to use 40µl, 80µl, and 160µl of CiT Viral Mix in separate wells. For the final calculation, use the samples that have 70%-85% RFP+ cells on day 4. A control lentiviral vector may be used before running the assay to test the transduction efficiency of your cells (see Additional Required Materials).

Day 1

Replace medium with fresh growth medium, grow cells under standard conditions for 3 days. Passage cells as needed. Cell should not become confluent.

Day 4

1. Analyze cells by flow cytometry, using settings below:

> **Channel 1:** excitation 488nM, emission 530/20nm (GFP)
> **Channel 2:** excitation 561nM, emission 590/20nm (RFP)

2. Calculate the mean GFP and mean RFP intensity for both parental and dCas9-Rep transduced cells, then calculate the relative GFP/RFP expression for each sample (normalized GFP intensity).

Transcriptional repression efficiency in the dCas9-Rep cell line is calculated as the ratio between the normalized GFP intensity of Parental Cells and the normalized GFP intensity of dCas9-Rep Cells.

> **Note:** The Calculated Transcriptional repression efficiency is an underestimation of the actual transcriptional repression, due to % of non-transduced cells.

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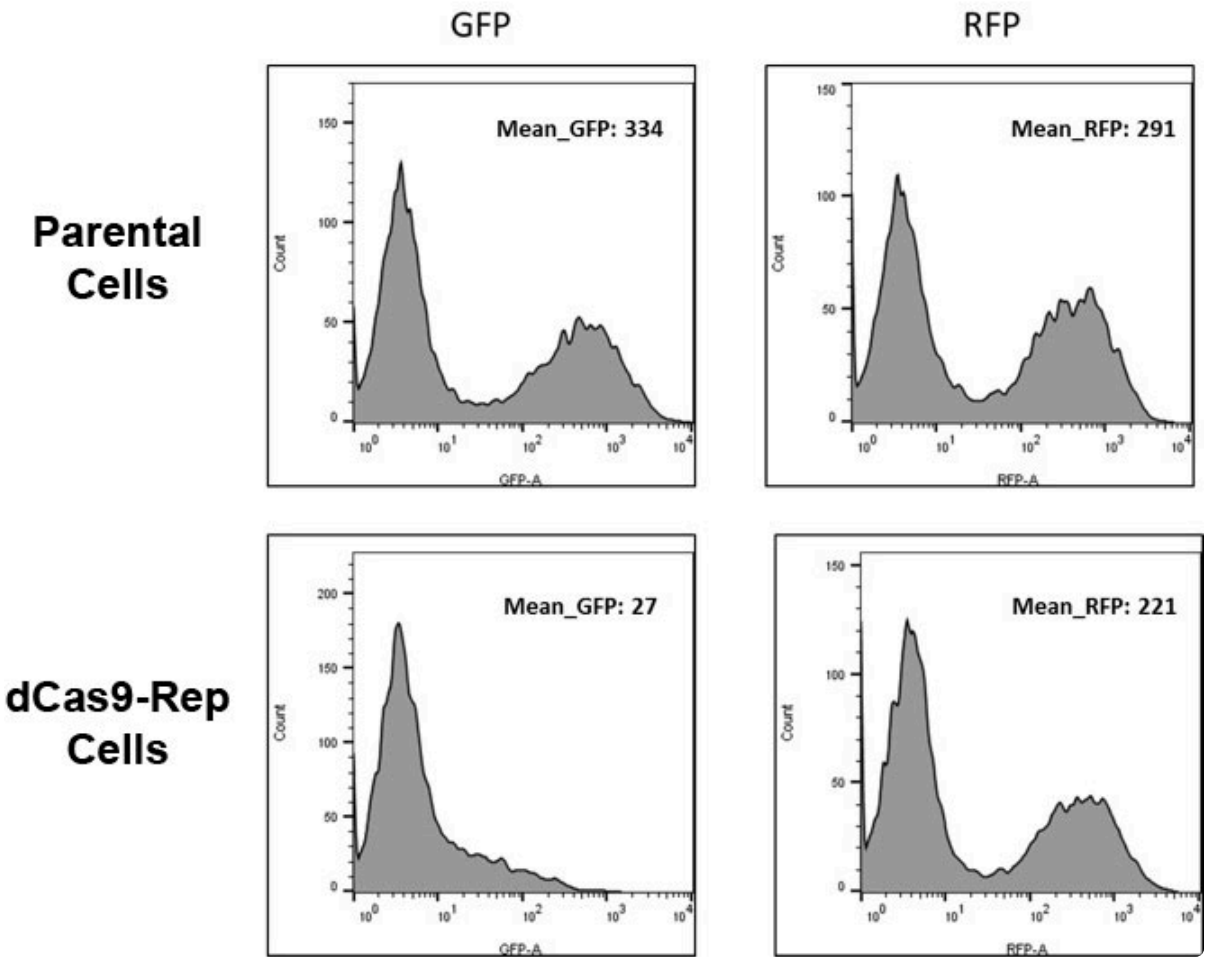

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4.2. dCas9-Rep Activity Calculation

Transcriptional repression efficiency of in the dCas9-Rep cell line is calculated as the ratio between the normalized GFP intensity of Parental Cells and the normalized GFP intensity of dCas9-Rep Cells.

The data above was calculated using the suggested positive control MDA-MB-231-dCas9-KRAB and parental MDA-MB-231 cells (see Additional Required Materials). The level of repression is calculated as follows using this data as an example:

	Mean_GFP	Mean_RFP	GFP/RFP
Parental	334	291	334 / 291 = 1.148
dCas9-KRAB	27	221	27 / 221 = 0.122

> **dCas9-Rep fold repression = 1.148 / 0.122 = 9.4 fold**

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5. Technical Support

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For the latest technical news and updates, visit Cellecta's blog at: https://cellecta.com/blogs/news

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6. Safety Guidelines

The HIV-based lentivector system is designed to maximize its biosafety features, which include:

- A deletion in the enhancer of the U3 region of 3'ΔLTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
- The tat-transactivator domain has been removed from the upstream 5'-LTR in the lentivector to disable transcription from the 5'-LTR.
- The three proteins (gag, pol, env) are necessary for replication and packaging of the lentiviral vector. When packaging lentiviral particles, the genes encoding these three packaging proteins are separately introduced and expressed in the packaging cells using separate ordinary plasmids that lack packaging signals and share no significant homology to the lentivector. As a result, none of the HIV-1 genes (gag, pol, env) will be present in the packaged lentiviral genome.
- None of the HIV-1 genes (gag, pol, env) will be present in the packaged lentiviral genome, as they are expressed from packaging plasmids lacking packaging signal—therefore, the lentiviral particles generated are replication-incompetent.
- Lentiviral particles will carry only a copy of your expression construct.

Despite the above safety features, use of replication-incompetent lentiviral particles falls within NIH Biosafety Level 2 criteria. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at:

> https://www.cdc.gov/labs/bmbl/

It is also important to check with the health and safety guidelines at your institution regarding the use of lentiviruses and follow standard microbiological practices, which include:

- Wear gloves and lab coat at all times when conducting the procedure.
- Always work with lentiviral particles in a Class II laminar flow hood.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leakproof, properly marked (biohazard, infectious waste) container and sealed for transportation from the laboratory.

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7. Contact Us

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