CELLECTA

USER MANUAL

CRISPRaTest™ Functional dCas9-Activator Assay Kit

v1 — Last update: 19 March 2024

Cellecta, Inc.

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1. CRISPRaTest™ Functional dCas9-Activator Assay Kit

The CRISPRaTest™ Functional dCas9-Activator Assay Kit (Cat.# CRATEST) is designed to measure the activity of dCas9 transactivator (e.g., dCas-VPH, dCas9-VPR) in most mammalian cell systems. The choice of kit CRISPRaTest™ or CRISPRaTest™ (EF1L), depends on the cell line to be tested. In iPS cells, where the CMV promoter used in the CRISPRaTest™ Kit is quickly silenced, the CRISPRaTest™ Kit (EF1L) is strongly recommended.

The CRISPR gene activation system (CRISPRa), a modification of the original CRISPR knockout system, uses a sgRNA molecule to direct a catalytic inactive Cas9 (dCas9) fused with a Transcriptional Activator (TA), to a target location on a gene promoter. The sgRNA-dCas9-TA complex then increases the expression levels of the gene transcribed from the targeted the promoter.

The CRISPRaTest Kit provides reagents to measure the activator strength of any *Streptococcus pyogenes* dCas9 transactivator (dCas9-TA) hybrid protein in cells designed to be used for CRISPRa experiments, using a simple FACS-based assay. The kit contains pseudoviral packaged lentiviral constructs with a green fluorescent protein (GFP) gene that increases in expression when expressed in cells with active dCas9-TA. The kit provides enough reagents to measure this activity in 5-15 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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Last modified: 15 March 2024

2. Kit Components

The CRISPRaTest™ comes in two formats:

- CRISPRaTest™ has the CMV promoter:

Component	Volume
CaT-Active (CaT-A) Viral Mix	500 µl
CaT-Background (CaT-B) Viral Mix	500 µl
Transduction Reagent (1000X)	50 µl

- CRISPRaTest™ (EF1L) has the EF1L promoter:

Component	Volume
CaT-Active (CaT-A EF1L) Viral Mix	500 µl
CaT-Background (CaT-B EF1L) Viral Mix	500 µl
Transduction Reagent (1000X)	50 µl

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

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3. Additional Required Materials

Use of the CRISPRaTest 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	Price
SVVPHC9B-PS	CRISPRa dCas9-VPH pRDVCCB-CMV-dCas9-VPH-2A-Blast (plasmid)	25 µg	$300
SVVPHC9B-VS	CRISPRa dCas9-VPH pRDVCCB-CMV-dCas9-VPH-2A-Blast (virus)	1×10^6 TU	$500

Cell Line

dCas9-VPH+ MDA-MB-231 Cells (Cat.# ZMDAMB231-CVPH, 10^6 cells) may be run in parallel with the user's dCas9-transactivator (dCas9-TA) cells as a positive control for the assay.

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	Price
SGCTL-NT-pRSG16-V	Non-Targeting CRISPR control pRSG16-U6-(sg)-UbiC-TagRFP-Puro Vector (virus)	1×10^7 TU	$500

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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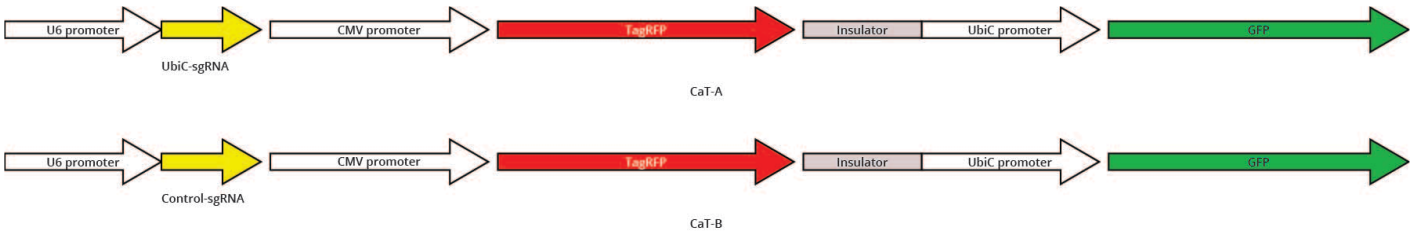
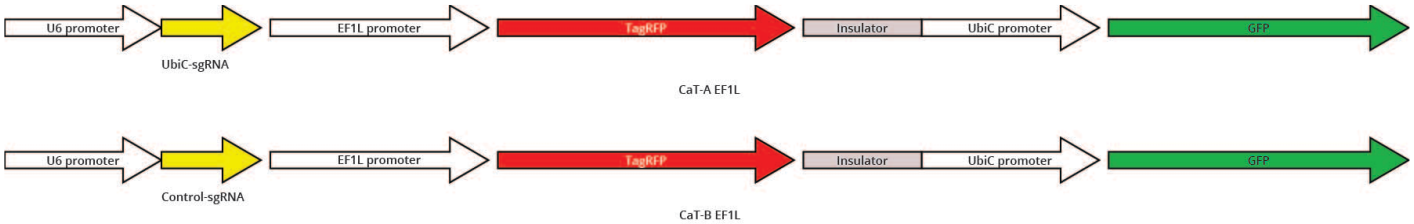
4. Protocol Overview

Both the CaT-Active (CaT-A) and CaT-Background (CaT-B) reagents contain lentiviruses that express RFP and GFP from separate transcripts, each driven by its own promoter. The RFP transcript is driven by the strong CMV or EF1L promoter, while GFP is driven by the weak UbiC promoter (see Figure below).



CaT-A and CaT-B with CMV lentiviral vector



CaT-A and CaT-B with EF1L lentiviral vector

The CaT-A lentivirus expresses a sgRNA targeting the UbiC promoter. When transduced into cells expressing a functional dCas9-TA fusion protein (such as Cellecta's dCas9-VPH), GFP expression increases in response to the sgRNA/dCas9-TA mediated transactivation of the UbiC promoter.

The CaT-B lentivirus expresses a control non-targeting sgRNA which does not recruit the dCas9-transactivator onto the UbiC promoter, so GFP expression is unaffected.

To assay cells for dCas9-TA activity, transduce two separate samples of the same dCas9-TA expressing cells with the CaT-A and CaT-B viruses. GFP fluorescence in the cells transduced to the CaT-A virus will be significantly increased as compared to the CaT-B cells. RFP fluorescence in both samples will be unaffected. The difference in the mean GFP fluorescence between the transduced cells of the two samples (normalized against the mean RFP fluorescence) provides a quantitative measure of the activity of the dCas9-TA transactivator in the dCas9-TA cells.

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4.1. Assay Procedure

Two populations of dCas9-TA cells are transduced with the CaT-A and CaT-B viral mix reagents, respectively. After 4 days, each population is analyzed by flow cytometry. The mean GFP and RFP fluorescent values of the transduced cells are then used to calculate dCas9-TA activity in the dCas9-TA cells.

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

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

Day 0

1. Quickly thaw the CaT-A and CaT-B lentiviral particles 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 -80°C, and used again for subsequent experiments.

2. Suspend dCas9-TA cells in growth medium supplemented with 1X Transduction Reagent, at a density of ca. 100,000 cells/ml.

> **Note:** This cell density was calculated for MDA-MB-231 cells. Depending on cell size and growth, you may need to use a different concentration and correspondingly-sized plate. As a rule of thumb, cells should be transduced at a density such that they would not become confluent before ~48 hours.

3. Aliquot 1 ml of cell suspension (100,000 cells) into each of 2 wells of a 12-well plate.

4. Add 20 µl of CaT-A virus into one well and 20 µl of CaT-B virus into the other well, mix and return cells to incubator.

> **Note:** For most cell lines, 20 µl of CaT viral reagents will suffice to obtain between 20%-50% RFP+ cells (the recommended % of transduced cells for optimal assay accuracy). For hard to transduce cell lines, more virus might be needed. In doubt, it is recommended to use increasing amounts of CaT-a and Cat-B viruses in separate transductions. For the final calculations, use the samples showing between 20%-50% RFP+ cells on day 4.

Day 1

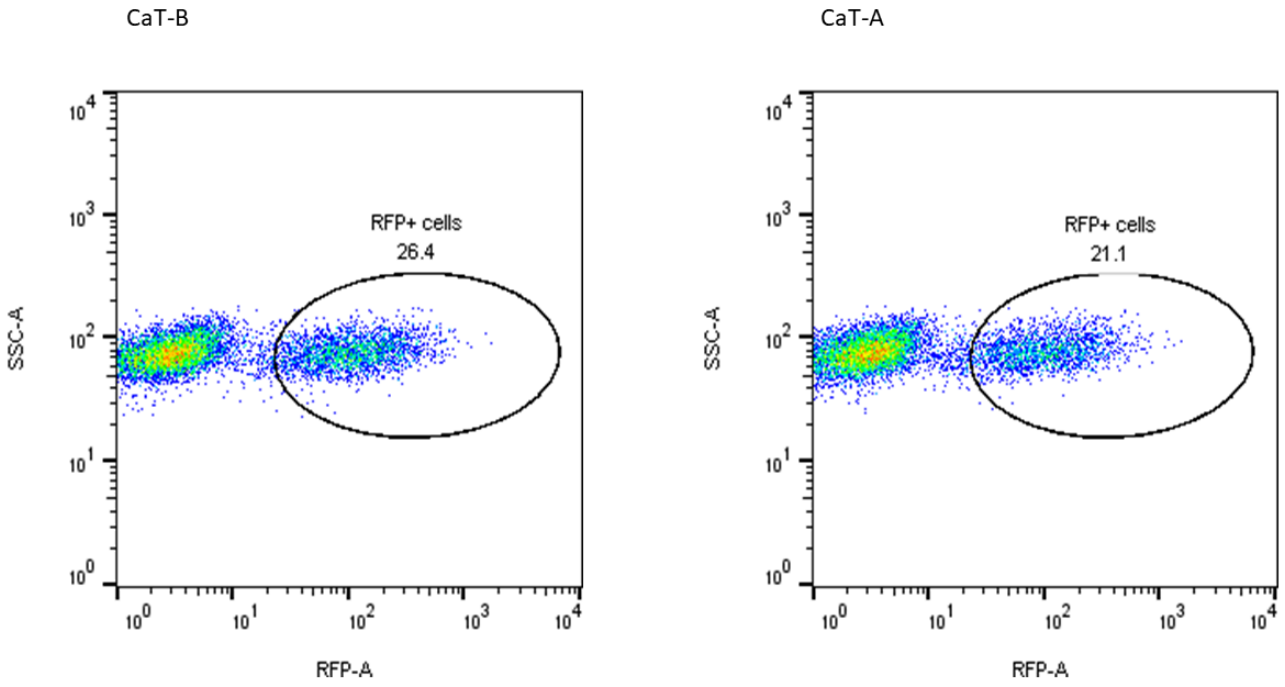
Exchange medium with fresh growth medium, grow cells under standard conditions for additional 3 days (passage cells as needed, cells should not become confluent).

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. For both CaT-B and CaT-A samples, gate in RFP+ cells as shown below:



3. Calculate the mean GFP and mean RFP intensity for CaT-B (RFP+ cells) and CaT-A (RFP+ subset), then calculate the relative GFP/RFP intensity for CaT-B (RFP+ cells) and CaT-A (RFP+ cells)

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4.2. dCas9-TA Activity Calculation

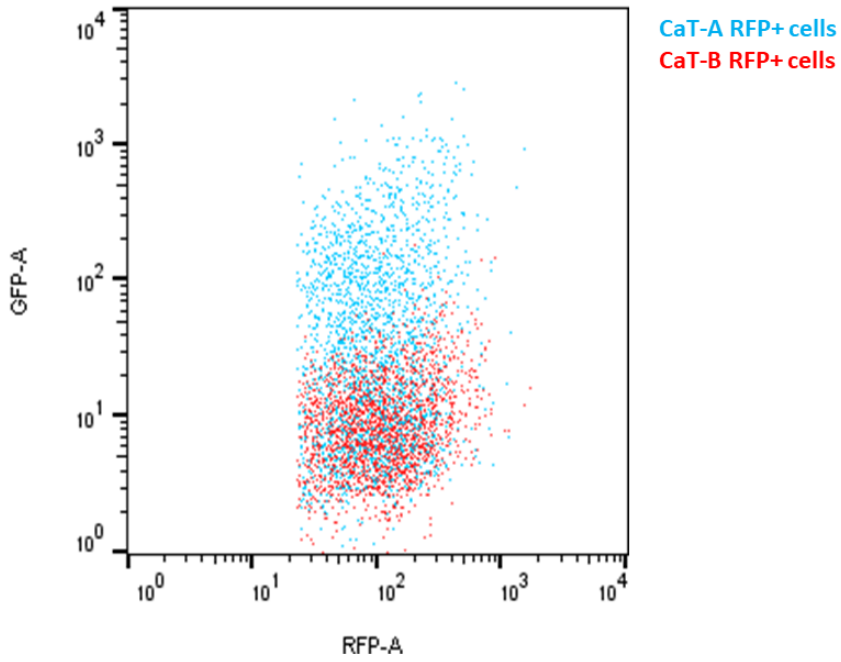
The fold activation of the UbiC promoter by dCas9-TA is calculated as the ratio between the GFP/RFP intensity of CaT-A (RFP+ subset) and the GFP/RFP intensity of CaT-B (RFP+ subset).

Below is an example calculation based on positive control MDA-MB-231-dCas9-VPH cells (see Additional Required Materials to obtain this cell line):

	Mean_GFP	Mean_RFP	Normalized GFP (GFP/RFP)
CaT-B (RFP+ subset)	11.2	133	11.2 / 133 = 0.084
CaT-A (RFP+ subset)	141	110	141 / 110 = 1.282

> **dCas9-TA induced fold induction = 1.282 / 0.084 = 15.3 fold**

The activation of the UbiC promoter by dCas9-TA can also be visualized by plotting and superimposing the GFP and RFP intensity of Cat-B (RFP+ subset) and Cat-A (RFP+ subset) samples, as shown below:



As an additional negative control (optional), the parental cell line of the dCas9-TA cells can be transduced with CaT-A and CaT-B viruses. For cells without dCas9-TA, the fold activation should be close to 1.

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5. Technical Support

Email Addresses

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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 RSV promoter upstream of 5'LTR in the lentivector allows efficient Tat-independent production of lentiviral RNA, reducing the number of genes from HIV-1 that are used in this system.
- Number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev). The corresponding proteins are expressed from different plasmids lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector to prevent generation of recombinant replication-competent virus.
- None of the HIV-1 genes (gag, pol, rev) 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 HIV-based vectors 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/biosafety/publications/bmbl5/index.htm

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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