



CELLECTA

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CRISPRtest™
Functional Cas9 Activity Kit
For Human Cell Lines

User Manual

Cat.# CRTEST

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Contents

I. Kit Overview.....	3
I.A. Intended Use.....	3
I.B. Background.....	3
I.C. CRISPRtest Kit Principle	4
I.D. Materials Provided.....	5
I.E. Required Materials Needed from Other Vendors	5
I.F. Related Products and Services from Collecta	5
II. CRISPRtest Protocol.....	6
II.A.Overview	6
II.A.1. Day 0 – Transduction.....	6
II.A.2. Day 1 – Media Change	6
II.A.3. Day 3 – CRISPRtest Dilution Selection.....	6
II.A.4. Day 10 – Read	7
II.A.5. Representative Data & Analysis.....	7
II.B.Alternative Protocol (<i>ONLY if 561 nm excitation not available</i>)	8
II.B.1. Day 0 – Transduction.....	8
II.B.2. Day 1 – Media Change	8
II.B.3. Day 3 – CRISPRtest Dilution Selection.....	8
II.B.4. Day 10 – Read	9
III. Other Information	10
III.A. Technical Support.....	10
III.B. Safety Guidelines.....	11
III.C. Terms and Conditions.....	12

I. Kit Overview

I.A. Intended Use

Collecta's CRISPRtest™ Functional Cas9 Activity Kit is an easy-to-use, highly sensitive kit for measuring functional Cas9 activity in human cell lines. It is intended as a companion product for high-throughput, CRISPR-based genetic screening or generation of isogenic knockout cell lines.

Please read the entire user manual before proceeding with your experiment.

I.B. Background

CRISPR/Cas9 system:

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes denoted as the CRISPR/Cas9 system is a targeted gene-editing tool adapted from *Streptococcus pyogenes* that enables the permanent knockout of target genes. Single Guide RNAs (sgRNA or gRNA) can be designed to direct the Cas9 nuclease to a chosen specific genomic sequence, upon which the Cas9 cleaves the target gene and permanently knocks it out (Figure 1).

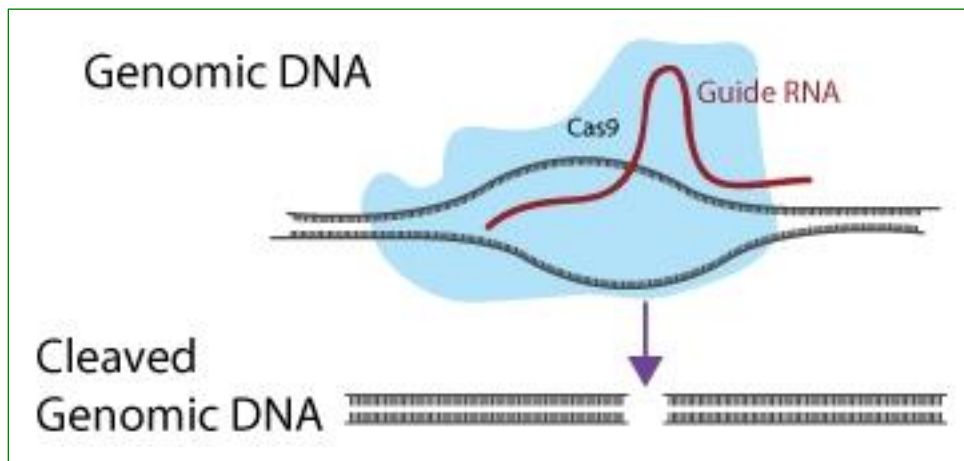


Figure 1. Guide RNA (sgRNA or gRNA) directs the Cas9 nuclease to a specific genomic sequence, upon which the Cas9 cleaves the target gene and permanently knocks it out.

I.C. CRISPRtest Kit Principle

Would you like to know if Cas9 is active in your cells? Collecta's CRISPRtest™ Functional Cas9 Activity Kit allows you to assess the level of Cas9 activity in your human cell line using two pre-mixed lentiviral-packaged vectors, the first containing a green fluorescent protein (GFP) marker and sgRNA sequence targeting an essential gene (human PCNA) and the second containing a red fluorescent protein (RFP) marker and a non-targeting sgRNA. Transduction of CRISPRtest virus into cells will result in an initial ratio of GFP cells : RFP cells. Upon cell growth in the presence of active Cas9, the ratio of GFP cells : RFP cells will decrease proportionally to Cas9 activity.

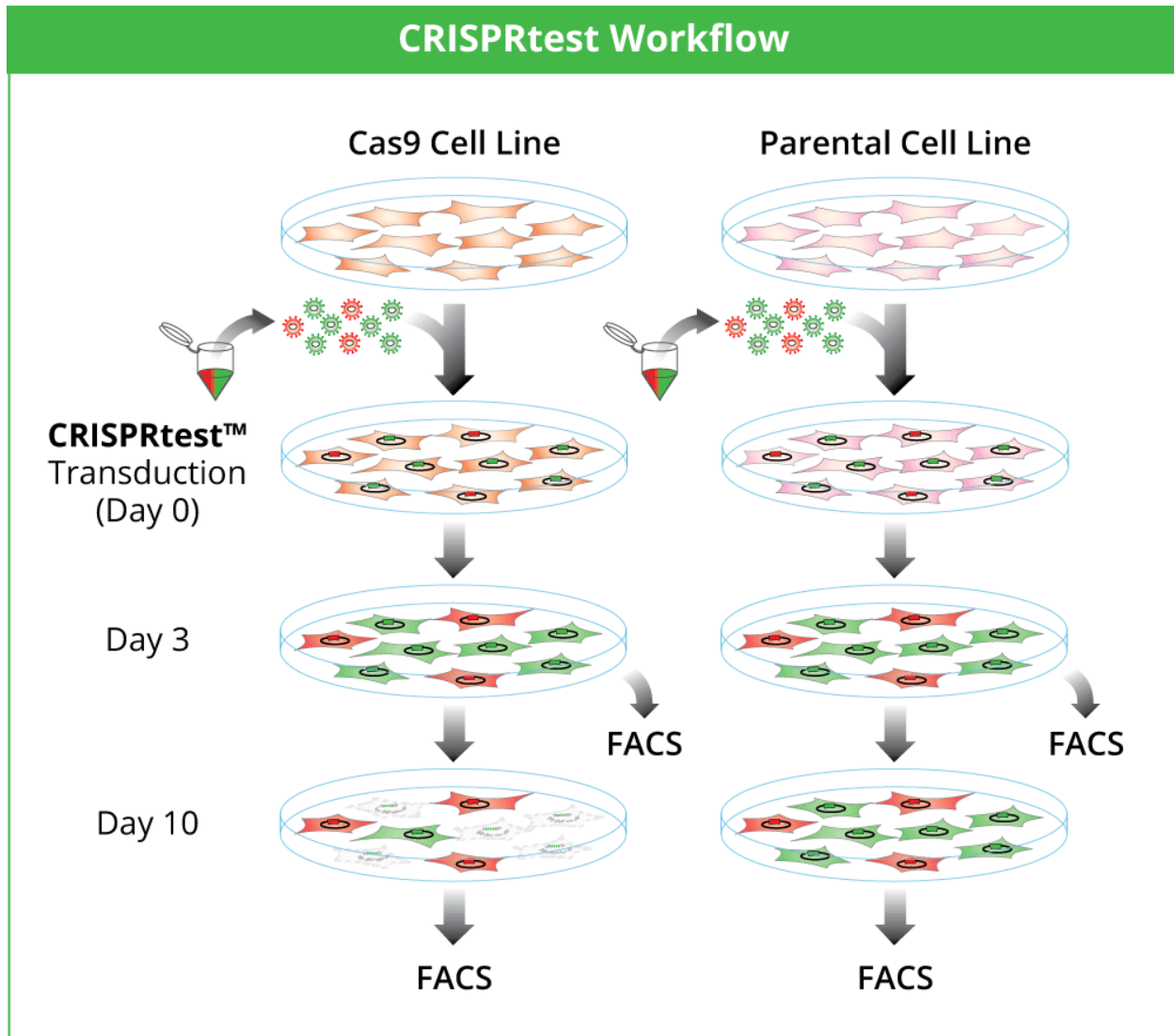


Figure 2. CRISPRtest workflow. Cas9 cell line and Parental cells are transduced with the CRISPRtest Virus. At Day 3 and Day 10 after transduction, a portion of the cells are analyzed by flow cytometry to determine GFP:RFP ratios. These ratios are then used to calculate the percentage knockout which is an assessment of Cas9 activity.

I.D. Materials Provided

The amount of reagents provided in the CRISPRtest kit is sufficient to test up to 5 pairs of human cell lines for Cas9 activity.

The kit contains the following components:

Description	Quantity	Storage
CRISPRtest™ Virus	500 µl, 1 vial	-80°C
Transduction Reagent (1000X)	50 µl, 1 vial	-80°C

I.E. Required Materials Needed from Other Vendors

- Complete cell culture medium
- Tissue culture plates and related tissue culture supplies

I.F. Related Products and Services from Cellecta

- CRISPRuTest™ Functional Cas9 Activity Assay Kit for Any Cell (Mammalian) (Cat.# CRUTEST)
- CRISPRiTest™ Functional dCas9-Repressor Assay Kit (Cat.# CRITEST)
- CRISPRaTest™ Functional dCas9-Activator Assay Kit (Cat.# CRATEST)
- LentiFuge™ Viral Concentration Reagent (Cat.# LFVC1)
- Cas9 expression vectors in plasmid and packaged formats ([click for more info](#))
- Custom and control lentiviral sgRNA constructs ([click for more info](#))

For more information, visit www.cellecta.com, email us at sales@cellecta.com, or call 650-938-3910.

II. CRISPRtest Protocol



CAUTION: You are working with infectious lentiviral particles at this stage. Please follow the recommended guidelines for working with BSL-2 safety class materials (see [Safety Guidelines](#)).

II.A. Overview

The following protocol uses packaged lentiviral particles for transduction into Cas9 and parental target human cells to determine Cas9 activity.

II.A.1. Day 0 – Transduction

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

1. Suspend parental cells and Cas9 cells in growth media supplemented with 1X CRISPRtest Transduction Reagent (dilute supplied Transduction Reagent 1000-fold in growth media), at a density of 100,000 cells/ml.
2. For each cell line, aliquot 1 ml of cell suspension per well in 4 wells of a 12-well plate.
3. Add 0 μ l, 3 μ l, 10 μ l, and 30 μ l of CRISPRtest Virus in the 4 different wells, mix, and incubate for 24 hours at 37°C, 5% CO₂.

II.A.2. Day 1 – Media Change

4. 24 hours post-transduction, change cell growth medium with fresh growth medium. Continue incubation in the CO₂ incubator for an additional 48 hours.

II.A.3. Day 3 – CRISPRtest Dilution Selection

5. Collect an aliquot of cells from each well and analyze by flow cytometry (FACS analysis) using the following settings:

Flow cytometer settings:

Channel 1: Excitation 488 nm, Emission ~530 nm (TagGFP2)

Channel 2: Excitation 561 nm, Emission ~600 nm (TagRFP)

6. For each sample, calculate the percentage of GFP(-)RFP(+) cells (%RFP) and the percentage of GFP(+)RFP(-) cells (%GFP).
7. For each cell line, choose the CRISPRtest Virus dilution (from Step 3) where the %RFP is between 10%-20% (it should be the same dilution for both cell lines), then discard the other dilutions.

NOTE: CRISPRtest Virus dilutions should give similar %RFP in both parental and Cas9 cell lines. At Day 3, the GFP:RFP ratio should be ~1 in both cell lines.

Calculations	Parental Cell Line (Day 3)	Cas9 Cell Line (Day 3)
%RFP	10%	10%
%GFP	12%	10%
GFP:RFP ratio	= 12 / 10 = 1.2	= 10 / 10 = 1.0

Table 1. Example of Flow Cytometry Readings. At Day 3, the GFP:RFP ratio is ~1 for both Parental and Cas9 cell lines, as expected. In this example, MDA-MB-231 cells expressing Cas9 were tested with the CRISPRtest kit.

- Grow the selected CRISPRtest dilutions for an additional 7 days. As cells must be in log phase for the whole duration of the experiment, passage the cells accordingly.

II.A.4. Day 10 – Read

- Analyze cells by flow cytometry, using the same settings as on Day 3.
- Calculate the GFP:RFP ratio in both cell lines (see Figure 3 and Table 2, below).
- Assess Cas9 activity using the following formula:

$$\%KO = 1 - [(GFP:RFP)_{Cas9} / (GFP:RFP)_{Parental}]$$

The calculated %KO value is the percentage of cells in the Cas9 cell line where the PCNA gene was knocked out (i.e., where CRISPR knockout was effective for both alleles). The closer this value is to 100%, the more active Cas9 is in the cells.

II.A.5. Representative Data & Analysis

The following is an example of data generated from a CRISPRtest experiment in MDA-MB-231 cells.

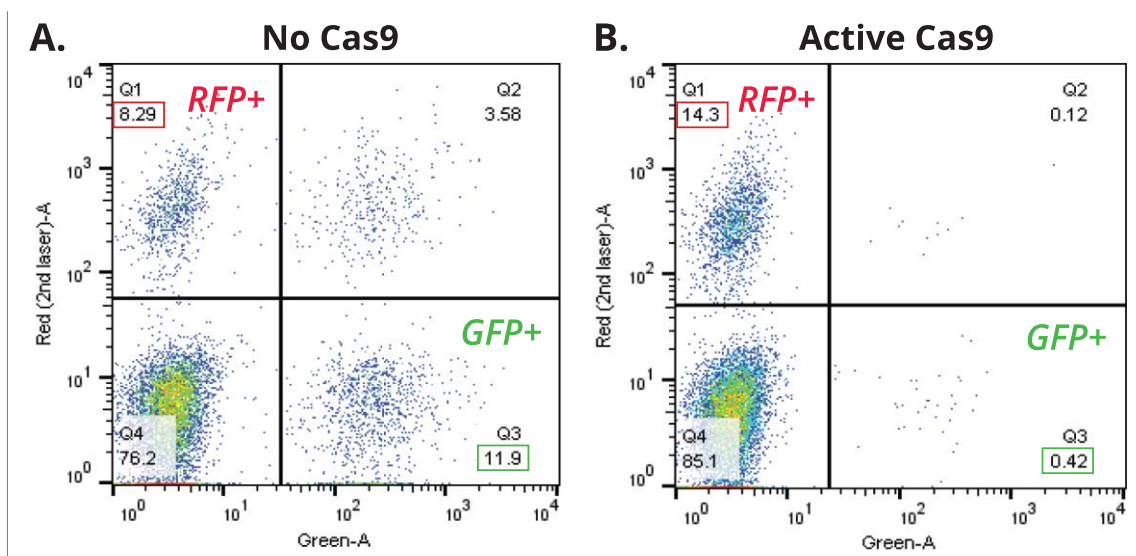


Figure 3. CRISPRtest experimental data, showing flow cytometry scatter plot analysis of GFP and RFP expressing cells at Day 10 in (A) Parental and (B) Cas9-expressing MDA-MB-231 cells. The percentages of RFP-positive cells

calculated in the GFP(-) RFP(+) quadrant Q1 and GFP-positive cells in the GFP(+) RFP(-) quadrant Q3 are used to calculate the percent knockout in the table below.

Calculations	Parental Cell Line (Day 10)	Cas9 Cell Line (Day 10)
%RFP	8.29%	14.3%
%GFP	11.9%	0.42%
GFP:RFP	11.9 / 8.29 = 1.44	0.42 / 14.3 = 0.03
%KO	1 - (0.03 / 1.44) = 98%	

Table 2. CRISPRtest results at Day 10. In Cas9 cells, 98% of cells that received the sgRNA targeting human PCNA experienced gene knockout in both PCNA alleles.

II.B. Alternative Protocol (**ONLY if 561 nm excitation not available**)

NOTE: This protocol should be used ONLY if you do not have the 561 nm (or 530 nm) laser. CRISPRtest can be used even if you do not have access to a flow cytometer that can detect TagRFP (Ex.: 561 nm / Em.: ~600 nm **or** Ex.: 530 nm / Em.: ~600 nm). The following protocol should be used if your flow cytometer can only detect TagGFP2 (Ex.: 488 nm / Em.: ~530 nm).

II.B.1. Day 0 – Transduction

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

1. Suspend parental cells and Cas9 cells in growth media supplemented with 1X CRISPRtest Transduction Reagent (dilute supplied Transduction Reagent 1,000-fold in growth media), at a density of 100,000 cells/ml.
2. For each cell line, aliquot 1 ml of cell suspension per well in 4 wells of a 12-well plate.
3. Add 0 µl, 3 µl, 10 µl, and 30 µl of CRISPRtest Virus in the 4 different wells, mix, and incubate for 24 hours at 37°C, 5% CO₂.

II.B.2. Day 1 – Media Change

4. 24 hours post-transduction, replace cell growth medium with fresh growth medium. Continue incubation in the CO₂ incubator for an additional 48 hours.

II.B.3. Day 3 – CRISPRtest Dilution Selection

5. Collect an aliquot of cells from each well and analyze by flow cytometry (FACS analysis) using the following settings:

Flow cytometer settings:

Channel 1: Excitation 488 nm, Emission ~530 nm (TagGFP2)

6. For each sample, determine the percentage of GFP(+) cells (%GFP).
7. For each cell line, choose the CRISPRtest Virus dilution (from Step 3) where the %GFP is in the range of 10%-20% (it should be the same dilution for both cell lines), then discard the other dilutions.
8. Grow the selected CRISPRtest dilutions for an additional 7 days. As cells must be in log phase for the whole duration of the experiment, passage the cells accordingly.

II.B.4. Day 10 – Read

9. Analyze cells by flow cytometry, using the same settings as on Day 3.
10. Calculate the %GFP in both cell lines.
11. Assess Cas9 activity using the following formula:

$$\%KO = 1 - [GFP_{Cas9} / GFP_{Parental}]$$

The calculated %KO value is the percentage of cells in the Cas9 cell line where the PCNA gene was knocked out (i.e., where CRISPR knockout was effective for both alleles). The closer this value is to 100%, the more active Cas9 is in the cells.

III. Other Information

III.A. Technical Support

Please contact us for any additional support or troubleshooting.

Phone / Fax

Phone (9am-6pm PST): +1 (650) 938-3910

Toll-Free (9am-6pm PST): +1 (877) 938-3910

Fax: +1 (650) 938-3911

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Sales: sales@collecta.com

General Information: info@collecta.com

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Mountain View, CA 94043

For the latest technical news and updates, visit Collecta's blog at:

<https://www.collecta.com/company/blog-news/>

To see the complete line of CRISPR-Test kits, visit our website:

<https://www.collecta.com/products-3/crispr-products/crisprtest-cas9-activity-assay-kits/>

III.B. 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) are 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 due to the potential biohazard risk of possible recombination with endogenous lentiviral sequences to form self-replicating virus or the possibility of insertional mutagenesis. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at:

http://www.cdc.gov/biosafety/publications/bmb15/bmb15_sect_iv.pdf

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.

III.C. Terms and Conditions

Cellecta, Inc. Limited License

Cellecta grants the end user (the "Recipient") of the CRISPRtest™ Functional Cas9 Activity Kit (the "Product") a non-transferable, non-exclusive license to use the reagents for internal research use only as described in the enclosed protocols; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Cellecta, Inc. - separate licenses are available for non-research use or applications. The Product is not to be used for human diagnostics or included/used in any drug intended for human use. Care and attention should be exercised in handling the Product by following appropriate research laboratory practices.

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Life Technologies Corporation End-User Label License for the use of Lentiviral Expression System:

"This product or service (based upon the Lentiviral Expression System) is sublicensed from Life Technologies Corporation under U.S. Patent Nos. 5,686,279; 5,834,256; 5,858,740; 5,994,136; 6,013,516; 6,051,427; 6,165,782; 6,218,187; 6,428,953; 6,924,144; 7,083,981 and 7,250,299 and corresponding patents and applications in other countries for internal research purposes only. Use of this technology for gene therapy applications or bioprocessing other than for nonhuman research use requires a license from GBP IP, LLC. Please contact GBP IP, LLC 537 Steamboat Road, Suite 200, Greenwich, CT 06830. Use of this technology to make or sell products or offer services for consideration in the research market requires a license from Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008."

Evrogen IP JSC End-User Label License for the use of lentiviral sgRNA constructs comprising TagRFP-encoded gene:

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