EpiQuik™ CRISPR/Cas9 Assay ELISA Kit (Colorimetric)

Base Catalog # P-4060

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ CRISPR/Cas9 Assay ELISA Kit (Colorimetric) is suitable for specifically measuring CRISPR-associated protein 9 (Cas9 and dCas9: *S. pyogenes)* amounts quantitatively with use of purified Cas9 nuclease and whole cell extracts isolated from tissues and cultured cells of various species.

Input Material: Input materials should be purified Cas9 nuclease or whole cell extract. The amount of whole cell extract for each assay can be between 0.5 µg and 5 µg with an optimal range of 1 to 2 µg. Total volume of the input material should not be more than 4 µl.

Whole Cell Extraction: You can use your method of choice for preparing whole cell extracts from the treated (transfected) and untreated (untransfected) samples. EpiGentek also offers a whole cell extraction kit (Cat. #OP-0003) optimized for use with this kit.

Whole cell extracts should be stored at −80°C in aliquots until use.

Internal Control: The standard control is provided in this kit for the quantification of Cas9 protein. Because content of Cas9 can vary in different samples, it is advised to run replicates for each sample to ensure that the signal generated is validated.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	48 Assays Cat. #P-4060-48	96 Assays Cat. #P-4060-96	Shipping Temperature	Storage Upon Receipt	Storage Checklist
WB (10X Wash Buffer)	14 ml	28 ml	Ambient	4°C	
CBB (Cas9 Binding Buffer)	6 ml	12 ml	Ambient	4°C	
DS (Developer Solution)	5 ml	10 ml	Ambient	4°C	
SS (Stop Solution)	5 ml	10 ml	Ambient	RT	
8-Well Assay Strips (With Frame)	6	12	Ambient	4°C	
Adhesive Covering Film	1	1	Ambient	RT	

Component	48 Assays Cat. #P-4060-48	96 Assays Cat. #P-4060-96	Shipping Temperature	Storage Upon Receipt	Storage Checklist
DAb (Detection Antibody, 1000X)*	6 µl	12 µl	Ice Pack	−20°C	
SI (Signal Indicator, 1000X)*	5 µl	10 µl	Ice Pack	−20°C	
ES (Enhancer Solution, 1000X)*	5 µl	10 µl	Ice Pack	−20°C	
Cas9 Standard Control (100 µg/ml)	5 µl	10 µl	Ice Pack	−20°C	

**For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.*

SHIPPING & STORAGE

The kit is shipped in two parts: one part at ambient room temperature, and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **DAb**, **SI**, **ES** and **Cas9 Standard Control** at −20°C away from light; (2) Store **WB**, **CBB**, **DS**, and the **8-Well Assay Strips** at 4°C away from light; (3) Store **all other components** at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

***Note**: (1) Check if wash buffer, **WB**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved; (2) check if a blue color is present in **DS** (Developer Solution), which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of **DS** required into a secondary container (tube or vial) before adding **DS** into the assay wells.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Adjustable pipette or multiple-channel pipette
- ☐ Multiple-channel pipette reservoirs

- ☐ Aerosol resistant pipette tips
- ☐ Microplate reader capable of reading absorbance at 450 nm
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Incubator for 37°C incubation
- ☐ Distilled water
- ☐ Whole cell extracts or purified Cas9 proteins
- ☐ Parafilm M or aluminum foil

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ CRISPR/Cas9 Assay ELISA Kit (Colorimetric) is tested against predetermined specifications to ensure consistent product quality. EpiGentek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

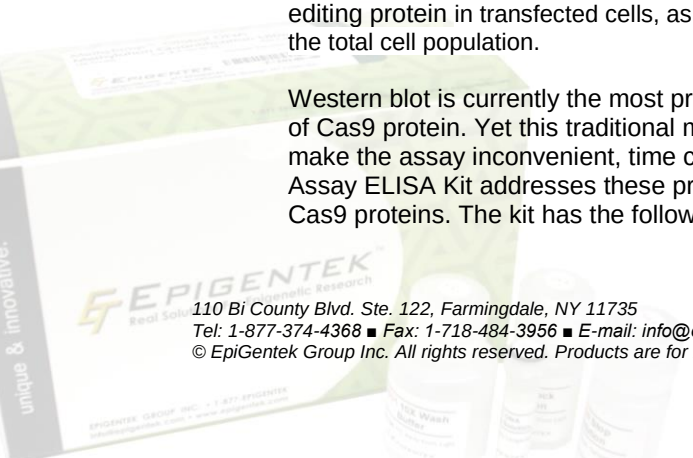
Product Updates: EpiGentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the kit when using that kit.

Usage Limitation: The EpiQuik™ CRISPR/Cas9 Assay ELISA Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

A BRIEF OVERVIEW

The discovery of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and Cas9 (CRISPR associated system or CRISPR associated protein 9 nuclease) found in bacteria to work as a defense mechanism against foreign DNA has proven to be an invaluable tool to target and modify a genetic sequence in gene editing and genome engineering applications. The system, known as CRISPR/Cas9, allows for sequence-specific cleavage of a targeted genomic locus by delivering the RNA-guided Cas9 nuclease and appropriate guide RNAs (gRNA) into a cell. In addition, Protospacer Adjacent Motif (PAM) sequence immediately following the specificity sequence is necessary for successful binding of the Cas9 nuclease. It is important and critical to monitor the level of Cas9 editing protein or track the Cas9 editing protein in transfected cells, as it will tell transfection efficiency and optimize the editing process in the total cell population.

Western blot is currently the most prominent assay technique for measuring the expression or amount of Cas9 protein. Yet this traditional method requires electrophoresis and transfer processes, which make the assay inconvenient, time consuming, and low throughput. The EpiQuik™ CRISPR/Cas9 Assay ELISA Kit addresses these problems by using a unique procedure to measure the amount of Cas9 proteins. The kit has the following features:



- Quick and efficient procedure, which can be finished within 3 hours.
- Innovative colorimetric assay in high throughput format to quantitatively measure Cas9 protein amount without the need for electrophoresis, especially suitable for screening Cas9-transfected clones.
- High sensitivity and specificity. The detection limit is as low as 0.1 ng/well with dynamic range of 0.5-20 ng/well within the indicated amount range of the whole cell extracts. Only recognizes Cas9 (*S. pyogenes).*
- The control is conveniently included for the quantification of Cas9 amount.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The EpiQuik™ CRISPR/Cas9 Assay ELISA Kit (Colorimetric) is designed for measuring Cas9 level in whole cell extracts or purified Cas9 proteins from various cells and tissues. In an assay with this kit, the Cas9 proteins in samples are tightly and stably spotted on the strip wells. The bound Cas9 proteins can then be recognized with detection antibody followed by a color development reagent. The ratio of Cas9 is proportional to the intensity of absorbance. The absolute amount of Cas9 can be quantitated by comparing to the Cas9 control.

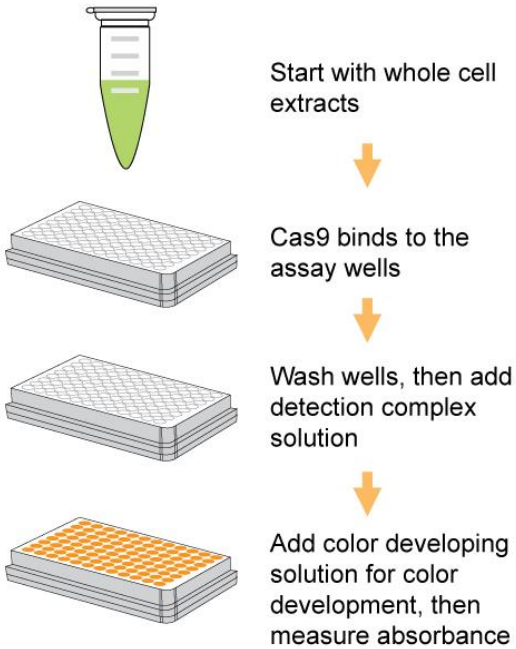


Fig 1. Schematic procedure of the EpiQuik™ CRISPR/Cas9 Assay ELISA Kit (Colorimetric).

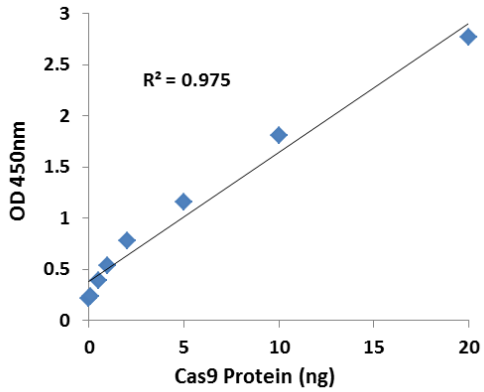


Fig 2. Illustrated standard curve.

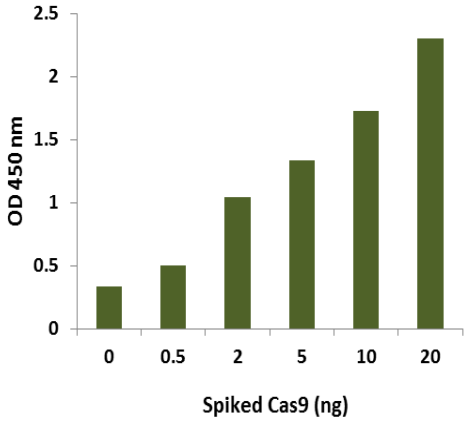


Fig 3. Cas9 proteins are spiked into Hela cell extracts at the different concentrations. The amount of the Cas9 proteins was measured using the EpiQuik™ CRISPR/Cas9 Assay ELISA Kit (Colorimetric).

ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input Amount: The amount of whole cell extract for each assay can be between 0.5 µg and 5 µg with an optimal range of 1 to 2 µg.

1. Working Buffer and Solution Preparation

a. Prepare **Diluted WB** (1X Wash Buffer):

48-Assay Kit: Add 13 ml of **WB** (10X Wash Buffer) to 117 ml of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit: Add 26 ml of **WB** (10X Wash Buffer) to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted WB** (1X Wash Buffer) can now be stored at 4°C for up to six months.

b. Prepare **Diluted Cas9 Standard Control**

Suggested Standard Curve Preparation: First, dilute **Cas9 Standard Control** to 50 ng/µl by adding 3 µl of **Cas9 Standard Control** to 3 µl of **CBB** (Cas9 Binding Buffer) and to 5 ng/µl by adding 1 µl of **Cas9 Standard Control** to 19 µl of **CBB**. Then, further prepare seven concentrations by using the 5 ng/µl and 50 ng/µl of **Diluted Cas9 Standard Control** with **CBB** into final concentrations of 0.1, 0.5, 1, 2, 5, 10, and 20 ng according to the following dilution chart:

Tube	Diluted Cas9 Standard Control (5 ng/µl)	Diluted Cas9 Standard Control (50 ng/µl)	CBB	Resulting Concentration
1	1.0 µl		49 µl	0.1 ng/µl
2	1.0 µl		9.0 µl	0.5 ng/µl
3	1.0 µl		4.0 µl	1 ng/µl
4	2.0 µl		3.0 µl	2 ng/µl
5	4.0 µl		0 µl	5 ng/µl
6		1.0 µl	4.0 µl	10 ng/µl
7		2.0 µl	3.0 µl	20 ng/µl

Note: *Keep each of the diluted solutions except* ***WB*** *(1X Wash Buffer) on ice until use. Any remaining diluted solutions other than* ***Diluted WB*** *should be discarded if not used within the same day.*

2. Cas9 Binding

a. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and positive controls) to ensure that the signal generated is validated. Carefully remove unneeded strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

b. Blank Wells: Add 100 µl of **CBB** to each blank well.

c. Standard Wells: Add 100 µl of **CBB** and 1 µl of **Diluted Cas9 Standard Control** to each standard well, each at a different concentration between 0.1 and 20 ng/µl (based on the dilution chart in Step 1b; see Table 2 under the "Suggested Strip Well Setup" section as an example).

d. Sample Wells: Add 100 µl of **CBB** and 1-4 µl of your samples.

Note: *Follow the suggested well setup diagrams.*

e. Tightly cover strip-well microplate with **Adhesive Covering Film** to avoid evaporation and incubate at 37°C for 120 min.

f. During the last 10 minutes of sample incubation, prepare the **Cas9 Detection Complex Solution**: In each 1 ml of **Diluted WB** add 1 µl of **DAb**, mix and then add 1 µl of **SI** and 1 µl of **ES**. Mix well.

Note: *The* ***Adhesive Covering Film*** *can be cut to the required size to cover the strips based on the number of strips to be used.*

g. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** (1X Wash Buffer) each time.

3. Detection Antibody Binding

a. Add 50 µl of the **Cas9 Detection Complex Solution** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 50 min.

b. Remove the **Cas9 Detection Complex Solution** from each well.

c. Wash each well four times with 150 µl of the **Diluted WB** each time.

Note: *Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.*

4. Signal Detection

a. Add 100 µl of **DS** to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The **DS** solution will turn blue in the presence of sufficient Cas9 product.

b. Add 100 µl of **SS** to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding **SS** and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

Note: *(1) Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice, once at 450 nm and once at 655 nm. Then, manually subtract the 655 nm ODs from 450 nm ODs; (2) If the strip-well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.*

5. Cas9 Calculation

a. Calculate the average duplicate readings for the sample wells and blank wells.

b. Calculate % Cas9 increase using the following formula if the samples are from treated and un-treated control tests:

$$Cas9\% = \frac{Transfected\ Sample\ OD - Blank\ OD}{Untransfected\ Control\ OD - Blank\ OD} \times 100\%$$

Example calculation:

Average OD450 of transfected sample is 0.9
Average OD450 of untransfected control is 0.3
Average OD450 of blank is 0.2

$$Cas9\% = \frac{0.9 - 0.2}{0.3 - 0.2} \times 100\% = 700\%$$

For detailed calculation:

1. Generate a standard curve and plot delta OD value (OD of each concentration point subtracts OD of blank) versus amount of **Cas9 Standard Control** at each concentration point.
2. Determine the slope as OD/ng (you can use Microsoft Excel statistical functions for slope calculation), then calculate the amount of Cas9 using the following formula:

$$Cas9\ (ng/ml) = \frac{(Sample\ OD - Blank\ OD)}{Slope \times sample\ amount\ (ul^*)} \times 1000$$

** Sample volume added into sample wells at Step 2d.*

SUGGESTED BUFFER AND SOLUTION SETUP

Table 1. Approximate amount of required buffers and solutions for defined assay wells based on the protocol.

Reagents	1 well	1 strip (8 wells)	2 strips (16 wells)	6 strips (48 wells)	12 strips (96 wells)
Diluted WB	2.5 ml	20 ml	40 ml	120 ml	240 ml
CBB	100 µl	800 µl	1600 µl	4800 µl	9600 µl
Cas9 Standard Control	N/A	N/A	4 µl (optional)	8 µl	8 µl
Cas9 Detection Complex	50 µl	400 µl	800 µl	2400 µl	4800 µl
Developer Solution	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
Stop Solution	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml

SUGGESTED STRIP WELL SETUP

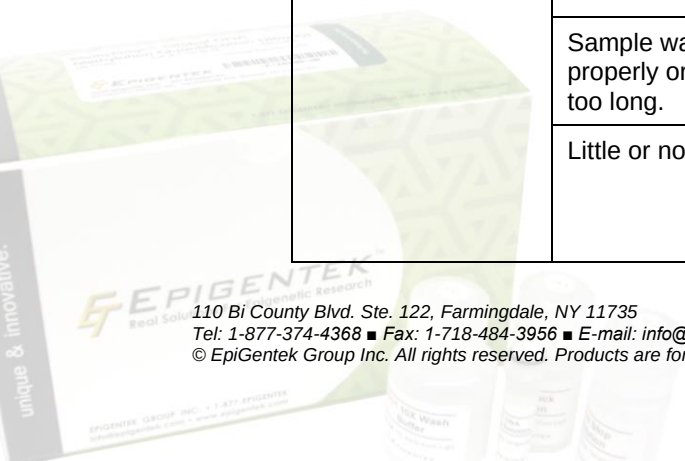
Table 2. The suggested strip-well plate setup for Cas9 protein quantification in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate. Strip 1 and Strip 2 are the control strips.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	SC 2 ng	Sample	Sample	Sample	Sample
B	Blank	SC 2 ng	Sample	Sample	Sample	Sample
C	SC 0.1 ng	SC 5 ng	Sample	Sample	Sample	Sample
D	SC 0.1 ng	SC 5 ng	Sample	Sample	Sample	Sample
E	SC 0.5 ng	SC 10 ng	Sample	Sample	Sample	Sample
F	SC 0.5 ng	SC 10 ng	Sample	Sample	Sample	Sample
G	SC 1 ng	SC 20 ng	Sample	Sample	Sample	Sample
H	SC 1 ng	SC 20 ng	Sample	Sample	Sample	Sample

Take Note! *(1) To reduce cross variation between replicates, it is important to load the wells in vertical formation according to the plate layout depicted above. (2) The standard controls should be assayed in parallel with the samples in the same plate. (3) For optimal binding and to reduce pipetting error, sample volume added should be 1 µl or more, but should not exceed 4 µl. (4) To ensure that* ***Cas9 Standard Control*** *and sample DNA are completely added into the wells, the DNA should be mixed well before use and the pipette tip should be placed into the* ***BS*** *solution in the well and aspirated in/out 1-2 times. Changing the tips each time when adding the sample will increase sample volume accuracy added into each well.*

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal or weak signal in both the positive control and sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order with the right amount, and if any steps in the protocol may have been omitted by mistake.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check if appropriate absorbance wavelength (450 nm) is used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperatures and the caps are tightly fastened after each opening or use.
No signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in Step 2c.	Ensure a sufficient amount of standard is added.
	The standard is degraded due to improper storage conditions.	Follow the Shipping & Storage guidance in this User Guide for storage of **Cas9 Standard Control.**
High background present in the blank wells	Insufficient washing of wells.	Check if washing recommendations at each step are performed according to the protocol.
	Contaminated by sample or standard.	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips.
	Incubation time with **DAb** is too long.	The incubation time at Step 3a should not exceed 90 min.
	Over-development of color.	Decrease the development time in Step 4a before adding **SS** Stop Solution in Step 4b.
No signal or weak signal only in sample wells	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of whole cell extracts or purified Cas9 proteins is used as indicated in Step 2d.
	Sample was not stored properly or has been stored for too long.	Ensure whole cell extract is stored in aliquots at proper temperature, for no more than 6 months.
	Little or no Cas9 in the sample.	This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared samples.



Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the guidance of washing and residue washing buffer is removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development solution or stop solution is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well H or from well 1 to well 12).

RELATED PRODUCTS

Protein Extract Preparation

OP-0002 EpiQuik™ Nuclear Extraction Kit
OP-0003 EpiQuik™ Whole Cell Extraction Kit

Cas9 Antibody

A-9000 CRISPR/Cas9 Monoclonal Antibody [7A9]