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Product Manual

Cas9 ELISA Kit

Cat. No. L01069

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I. DESCRIPTION

CRISPR-Cas9 is a revolutionary genome-editing system that enables precise DNA modification through RNA-guided double-strand breaks. As the molecular scissors in this system, the Cas9 endonuclease allows targeted gene deletion, insertion, or replacement across diverse biological models.

The GenScript Cas9 ELISA Kit (Cat. No. L01069) provides a rapid and quantitative solution for detecting *Streptococcus pyogenes* Cas9 (SpCas9) protein in cell lysates, viral vectors, and gene-editing reagents. This measurement is critical for optimizing CRISPR experimental workflows and ensuring quality control in therapeutic development.

This ELISA kit provides broad compatibility with commonly used Cas9 variants, including high-specificity mutants and constructs with single or double nuclear localization signals.

In addition to detecting wild-type Cas9, the kit is validated for multiple commonly used Cas9 constructs with different NLS configurations and enhanced specificity mutations, making it **highly versatile** for various experimental and therapeutic contexts.

II. ASSAY PRINCIPLE

Cas9 ELISA Kit utilizes two Cas9 monoclonal antibodies that bind to different epitopes of the Cas9 protein. When standards or samples are added to the capture plate, the Cas9 protein in the sample binds to the immobilized capture antibody. Then, the Cas9 detection antibody conjugated with biotin is added to interact with the Cas9 protein bound on the plate. Streptavidin-Horseradish Peroxidase conjugate (Streptavidin-HRP) is then added to bind to the biotin conjugated anti-Cas9 antibody. After the washing steps, 3,3',5,5'-Tetramethylbenzidine (TMB) solution is added, producing a blue color. The reaction is stopped by adding Stop Solution, which changes the color from blue to yellow. The intensity of the color can be read at 450 nm (with a reference at 650 nm) using a microplate reader. The amount of Cas9 protein in the sample is quantitatively determined based on a standard curve.

III. KIT CONTENTS

The kit provides the following reagents and items.

Table 1. Components of the kit

Component	Quantity/Size	Part No.
Cas9 Capture Plate	1 plate	B2-80
Cas9 Standard Stock	1 vial (50 µL)	B2-10
Biotin Anti-Cas9 Antibody	1 bottle (12 mL)	B2-20
Streptavidin-HRP	1 bottle (12 mL)	B2-30
Sample Dilution Buffer	1 bottle (60 mL)	B2-60
20× Wash Solution	1 bottle (60 mL)	B-A1-71
TMB Solution	1 bottle (12 mL)	B-A1-40
Stop Solution	1 bottle (6 mL)	B-A1-50
Plate Sealer	2 pieces	/

- Capture Plate: 96-well microplate (8 wells × 12 strips); 12 strips are configured in plate; the plate is sealed in a foil pouch with a desiccant.

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- Standard Stock contains 3.2 µg/mL of Cas9 protein (Recombinant Cas9 with a BPNLS at the N-terminus and a nucleoplasmin NLS at the C-terminus expressed by *E.coli*）
- GenScript offers the 20× Wash Solution (GenScript, B00063) if the customer requires additional wash solution.
- The kit does not include a paper manual. Please download the latest version of the manual from the GenScript official website.

IV. STORAGE

The unopened kit is stable for at least 12 months from the date of manufacture at 2°C to 8°C, and the opened kit is stable for up to 1 month from the date of opening at 2°C to 8°C.

V. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

- Appropriate sample preparation reagents (e.g., lysis buffer), depending on sample type

  Note: Optimal lysis conditions vary depending on sample source (e.g., mammalian cells, bacterial cultures, or viral particles). The buffer must ensure complete Cas9 protein release while maintaining epitope integrity for antibody recognition.

- Microplate reader capable of measurement at 450 nm and 650 nm.
- Data analysis and graphing software. It is recommended to use software that is capable of generating a four-parameter logistic (4-PL) curve-fit
- Automated microplate washer
- Deionized or distilled water
- Graduated cylinder
- Plastic container
- Tubes to aliquot and dilute samples
- 10 µL, 200 µL, and 1000 µL precision pipettes and pipette tips
- Multichannel pipettes
- Disposable reagent reservoir
- Absorbent paper
- Laboratory timer
- Refrigerator
- Centrifuge
- 25± 2°C and 37± 2°C incubator
- Rotary shaker

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- Vortex Mixer

VI. PRECAUTIONS

1. Any materials that may be contaminated with potentially infectious materials should be treated as infectious materials in accordance with local regulations. For more detailed information, please contact your local lab safety committee.
2. Reagents that contain preservatives may be toxic if ingested, inhaled, or spilled on skin.
3. Avoid contact with skin, eyes, or clothing with Stop Solution or TMB Solution. Keep the container tightly closed. In case of an accident, please seek medical advice immediately.
4. Do not use the kit if there is any visible damage to the packaging or kit contents.
5. Do not mix components from different batches. Do not mix with components from other manufacturers.
6. Do not use reagents beyond the stated expiry date.
7. All reagents must be equilibrated to room temperature (20-25°C) before running the assay. Only take an appropriate amount of reagents at once. Do not put unused reagents back into the vials as reagent contamination may occur.
8. Before opening the Standard Stock, quickly spin the vial to ensure that all the liquid has collected at the bottom, and prevent the liquid from splashing when opening the lid.
9. Use only distilled or deionized water and clean glassware.
10. Do not let wells dry during the test; add reagents immediately after completing washing steps.

VII. SPECIMEN COLLECTION AND STORAGE

1. The handling and storage information provided here is intended to be used as a general guideline. Sample stability has not been evaluated. When samples need to be stored for a long time, users need to evaluate the stability of the samples. It is the responsibility of the individual laboratory to use all available references and/or its studies when establishing alternate stability criteria that meet its needs.
2. Store specimens at -20°C or lower if not tested immediately. Avoid repeated freeze-thaw cycles.

VIII. PROTOCOL

- **Sample preparation**

  The sample preparation method is flexible and can be adapted based on the sample type.

  For cell samples, wash the cells with DPBS buffer at first. Centrifuge 1×10^6 of cells at 400 rpm for 10 minutes at 4°C. Add 400 µL of lysis buffer to the cell pellet and incubate for 10 minutes at 4°C. Centrifuge for 10 minutes at 12,000 rpm to precipitate the cell pellet. Decant the supernatant to a fresh tube and discard the cell pellet.

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For virus samples, it is recommended to add RIPA lysis buffer at 10% of the sample volume and lyse on ice for 10 minutes. After the lysis step, dilute the samples according to the estimated concentration to fall within the standard curve range.

Note: The lysis procedures described above are for reference only. This kit does not include lysis buffer, and customers should choose the appropriate lysis buffer and optimize the lysis steps according to their specific sample type.

- **Reagent Preparation**

All reagents must be equilibrated to room temperature before use (20-25°C). All samples and reagents should be vortexed before use. Store all reagents back in the refrigerator promptly after use.

1× Wash Solution: Dilute the 20× Wash Solution with deionized or distilled water with a volume ratio of 1:19. For example, dilute 40 mL of 20× Wash Solution with 760 mL of deionized or distilled water to make 800 mL of 1× Wash Solution. Store the solution at 2-8°C when not in use.

Note: If any precipitate is found in the 20× Wash Solution, incubate the bottle in a water bath (up to 50°C) with occasional mixing until all the precipitate is dissolved.

Standard Preparation: Centrifuge Standard Stock at 10,000 rpm for several seconds. Dilute Standard Stock with Sample Dilution Buffer with a volume ratio of 1:400. For example, mix 2.5 µL of Standard Stock with 997.5 µL of Sample Dilution Buffer to make 1 mL of 8 ng/mL Standard working solution with label of "Std-01". The recommended dilution gradient standards should be prepared with Sample Dilution Buffer to generate eight Cas9 concentrations recommended in **Table 2**: Sample Dilution Buffer (NC, Negative Control), 0.125, 0.25, 0.5, 1, 2, 4, and 8 ng/mL.

Table 2. Recommended standard preparation

Standard ID	Final Conc. (ng/mL)	Dilution Factor	Source	Source Volume (µL)	Matrix Volume (µL)	Final Volume (µL)
Std1	8	400	Standard Stock	2.5	997.5	1000
Std2	4	2	Std1	500	500	1000
Std3	2	2	Std2	500	500	1000
Std4	1	2	Std3	500	500	1000
Std5	0.5	2	Std4	500	500	1000
Std6	0.25	2	Std5	500	500	1000
Std7	0.125	2	Std6	500	500	1000
NC	/	0	/	/	1000	1000

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- **Capture Plate Preparation**

It is recommended that all standards and samples be prepared in duplicate at least. **Table 3** is an example of the setup of standards and samples.

Count the strips according to the number of test samples and install the strips. Make sure the strips are tightly snapped into the plate frame.

Leave the unused strips in the foil pouch and store at 2-8°C. The strips must be stored in the closed foil pouch to prevent moisture from damaging the Capture Plate.

Table 3. Setup of standards, quality controls, and samples on the Capture Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std1	Std1	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	Std2	Std2	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	Std3	Std3	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	Std4	Std4	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	Std5	Std5	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	Std6	Std6	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	Std7	Std7	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	NC	NC	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Std: Standard number; S: Sample number; NC: Negative Control

- **Test Procedure**

Standards and Samples Incubation

1. Add 100 µL of standard working solutions and samples to the corresponding wells in the Capture Plate.
2. Cover the plate with a Plate Sealer and incubate at 37°C on a rotary shaker at 300 rpm for 60 minutes.
3. Remove the Plate Sealer, discard the liquid, and wash the plate five times with 260 µL of 1× Wash Solution per well.
4. Tap the inverted plate onto absorbent paper to remove residual liquid in the wells after the washing steps.

Detection Antibody Incubation

5. Add 100 µL of Biotin Anti-Cas9 Antibody to all the testing wells.
6. Cover the plate with a Plate Sealer and incubate at 37°C on a rotary shaker at 300 rpm for 60 minutes.
7. Remove the Plate Sealer, discard the liquid, and wash the plate five times with 260 µL of 1× Wash Solution per well.

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8. Tap the inverted plate onto absorbent paper to remove residual liquid in the wells after the washing steps.

Enzyme Conjugate Incubation

9. Add 100 µL of Streptavidin-HRP to all the testing wells.
10. Cover the Plate with Plate Sealer and incubate at 37°C on a rotary shaker at 300 rpm for 10 minutes.
11. Remove the Plate Sealer, discard the liquid, and wash the plate five times with 260 µL of 1× Wash Solution per well.
12. Tap the inverted plate onto absorbent paper to remove residual liquid in the wells after the washing steps.

Substrate Reaction and Absorbance Measurement

13. Add 100 µL of TMB Solution to each well and incubate the plate in the dark at 25°C for 15 minutes without shaking (start timing after the addition of TMB Solution to the first well).

    Note: The operator has the option to extend or shorten the color development time based on their instrument parameters.

14. Add 50 µL of Stop Solution to each well to stop the reaction.
15. Read the absorbance in a microplate reader at dual wavelengths of 450 nm and 650 nm immediately. Then the absorbance at 650 nm, which serves as the reference, is subtracted from the absorbance of the same well measured at 450 nm to eliminate the background signal that comes from turbidity.
16. Plot the standard curve with the Cas9 concentration (ng/mL) on the x-axis and the corresponding mean absorbance value on the y-axis.
17. Using linear regression or a four-parameter logistic (4-PL) model, calculate the best-fitting curve through the points of the standard curve.

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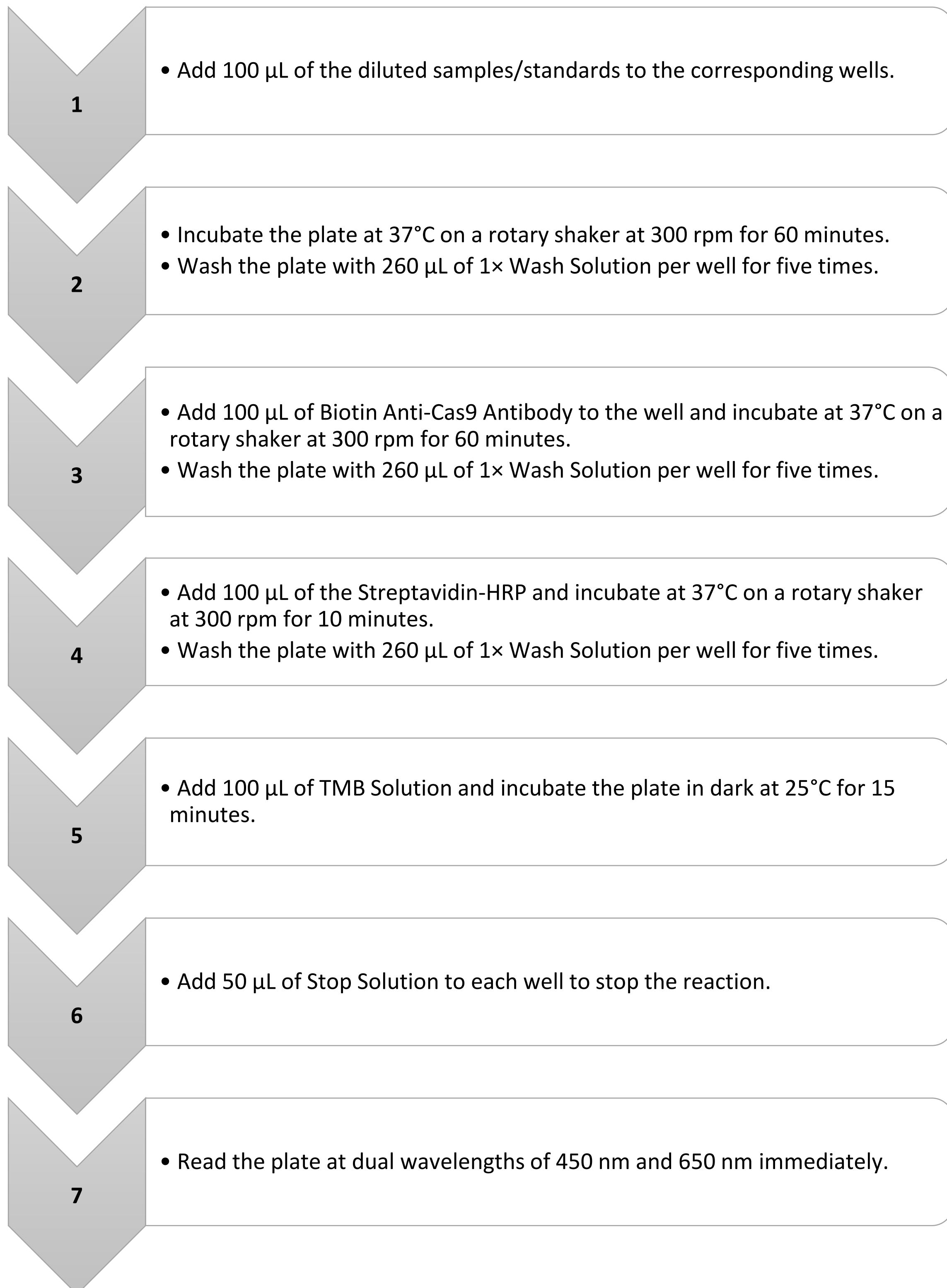
IX. ASSAY PROCEDURE SUMMARY



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X. INTERPRETATION OF RESULTS

- **Suggested Calculation of Data**

Statistical software can be used to create the standard curves. Choose a method with high Goodness of Fit (R^2) to analyze the data, such as linear regression or a four-parameter logistic (4-PL) model that provides point-to-point curve fitting.

The standard curve is for demonstration purposes only. It should be prepared each time an assay is performed.

Table 4. A typical sample of data for the Cas9 standard curve

Cas9 (ng/mL)	Absorbance (OD_{450}-OD_{650})		
	Duplicate 1	Duplicate 2	Average
8	2.579	2.626	2.603
4	1.454	1.380	1.417
2	0.759	0.816	0.788
1	0.391	0.444	0.418
0.5	0.240	0.201	0.220
0.25	0.129	0.134	0.131
0.125	0.075	0.075	0.075
0	0.026	0.027	0.027

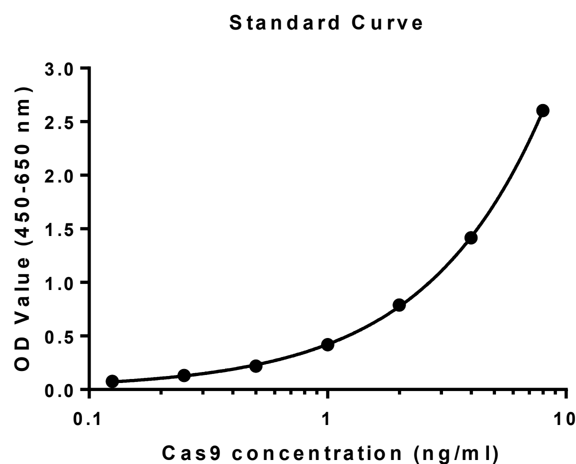

Figure 1. Typical Cas9 standard curve fitted with a four-parameter logistic model.

XI. ANALYTICAL PERFORMANCE

- **Assay Working Ranges**

The typical detection range of this kit for Cas9 is from 0.125 to 8 ng/mL according to the evaluation method recommended in ICH Q2(R2) [1].

- **Detection Capability**

According to the evaluation method recommended in ICH Q2 (R2) [1] and ICH-M10 [2], the LLOQ (Lower Limit of Quantification) of this kit is 0.125 ng/mL, and the ULOQ (Upper Limit of

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Quantification) is 8 ng/mL.

Note: Lower Limit of Quantification (LLOQ) is the lowest amount of an analyte in a sample that can be used for quantification. The upper Limit of Quantification (ULOQ) is the highest amount of an analyte in a sample that can still be used for quantification. 0.125 ng/mL is the lowest quantifiable concentration in our standard curve. Customers are required to verify the appropriate LLOQ for their samples.

- **Versatile Detection**

This kit is designed for the detection of a wide range of Cas9 proteins, including wild-type SpCas9, high-fidelity mutants (e.g., eSpCas9), and variants featuring nuclear localization signals (NLS) at the N-terminus, C-terminus, or both. Several commercially available Cas9 constructs have been tested, and the typical assay working ranges are presented in Table 5 and Figure 2. While not all Cas9 variants have been individually validated, the assay demonstrates broad compatibility and is likely to detect many commonly used formats. Users are encouraged to follow the standard protocol to assess compatibility with their specific Cas9 variant or construct.

Table 5. Typical assay working ranges for different Cas9 variants

Cat. No.	Cas9 variants	Typical assay working ranges (ng/mL)
Z03621	NLS - SpCas9	0.125-8
Z03702	NLS - SpCas9-NLS	0.125-8
Z03692	NLS - eSpCas9	0.125-8
Z03622	NLS - eSpCas90NLS	0.125-8

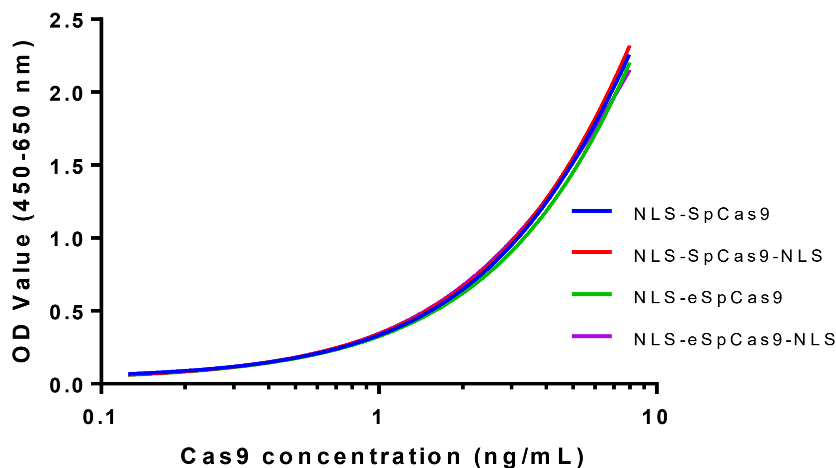

Figure 2: Cas9 variants curves

- **Measurement Precision**

Intra-assay and inter-assay precision were measured in 3 different concentrations of Cas9 samples, using 3 batches of kits.

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Table 6. Intra-assay and inter-assay precision of the kit

Theoretical Conc. (ng/mL)	Intra-assay (n=10)			Inter-assay (n=30)		
	Average Measured Conc. (ng/mL)	CV	Accuracy	Average Measured Conc. (ng/mL)	CV	Accuracy
6	5.813	5.3%	97%	5.863	7.0%	98%
1.5	1.503	4.1%	100%	1.471	6.6%	98%
0.375	0.383	4.9%	102%	0.376	6.8%	100%

- **Recovery**

In a recovery experiment, a known amount of Cas9 is added to the buffer matrix, and then the Cas9 ELISA Kit is used to measure the recovery (concentration) of the buffer matrix. Extreme pH, high salt concentrations, certain high protein concentrations, and certain detergents may result in inadequate recovery. The user can dilute the standards provided in this kit with the sample matrix of interest and perform recovery experiments to identify whether the sample can be accurately recovered in the sample matrix. This is necessary to determine the accuracy.

Table 7. Recovery rate of the kit

Sample ID	Theoretical Spike Conc. (ng/mL)	Measured Spike Conc. (ng/mL)	Spike Recovery Rate
Sample 1	0.250	0.240	96%
Sample 2	0.829	0.712	87%
Sample 3	1.000	1.071	107%
Sample 4	1.228	1.222	99%
Sample 5	1.273	1.108	87%
Sample 6	1.419	1.324	93%
Sample 7	1.804	1.689	94%
Sample 8	2.272	2.273	100%
Sample 9	2.379	2.230	94%
Sample 10	4.000	3.848	96%

- **Hook Effect**

When the sample concentration increased from 8 ng/mL to 80 ng/mL (Table 8), there was no hook effect below the 80 ng/mL concentration.

Table 8. Hook effect analysis of the kit

Sample ID	Cas9 (ng/mL)	Absorbance (OD_{450}-OD_{650})		
		Duplicate 1	Duplicate 2	Average
Hook 1	80	5.385	5.936	5.660
Hook 2	32	4.804	4.942	4.873
Hook 3	16	3.392	3.428	3.410
Std1	8	2.622	2.649	2.635

- **Linearity of Dilution**

The accuracy of the samples, which were diluted in a 3-fold gradient across 4 concentrations from a high-concentration sample for the analysis of dilutional linearity (Table 9).

Table 9. Dilutional linearity analysis of the kit

Dilution Factor	Conc. (ng/mL)	Average Detected Conc. (ng/mL)	CV	Accuracy
3	5.333	5.217	3.70%	98%
9	1.778	1.708	6.50%	96%
81	0.593	0.569	4.90%	96%
243	0.198	0.201	1.80%	102%

XII. TROUBLESHOOTING

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the washing apparatus works properly and wells are dry after aspiration.
	Wells are scratched with pipette tips or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples.	Remove any particulates by centrifugation before the assay
Weak/No Signal	Substrate is not added or added at the wrong time	Follow the manual to add the substrate properly
	Components are used from other lots or sources	Use only lot-specific components
	The substrate is contaminated	Use a new substrate from the same lot.
	Volumes of reagents are not correct.	Repeat the assay with the required volumes as noted in the manual.
	The plate is not incubated for the proper time or temperature	Follow the manual to repeat the assay
	The plate is not read within the specified time range	Read the plate within 5 minutes
High Background	Plate is not washed properly	Make sure the washing apparatus works properly
	The substrate is	Use a new substrate from the same

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	contaminated	Lot
	Evaporation of wells during incubations	Perform incubation steps with a plate sealer in a repeat assay.
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay.

XIII. REFERENCES

1. ICH: Q2(R2) Validation of Analytical Procedures
2. ICH: M10 Bioanalytical Method Validation and Study Sample Analysis

For research use only. Not intended for human or animal clinical trials, therapeutic or diagnostic use.

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