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Product Manual

His Tag ELISA Detection Kit

(384-Well Plate)

Cat. No. L01010

For Research Use Only. Not for Use in Diagnostic Procedures.

The operator should read the technical manual carefully before using this product.

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I. DESCRIPTION

The His tag, consisting of successive histidine (H) residues, primarily exists in three forms: HHHHHH (6x His), HHHHH (5x His), and HHHH (4x His). Due to its small size, less interference in protein folding, and low immunogenicity, His tag is the most prevalent tag used in recombinant protein expression. The DNA sequence encoding the His tag is typically incorporated at the N-terminus or C-terminus of various expression plasmids. Since His-tagged proteins have a high affinity for Ni²⁺ ions, Ni²⁺-resin chromatography can be used to efficiently purify His-tagged proteins from bacterial, yeast, and mammalian cell expression systems. Furthermore, anti-His tag antibodies are valuable tools for identifying His-tagged proteins through techniques such as western blotting, immunoprecipitation, and flow cytometry.

GenScript His Tag ELISA Detection Kit (384-Well Plate) offers a rapid, One-hour competition ELISA for the high-throughput, semi-quantitative detection of His-tagged proteins. This kit has several potential applications:

- Quickly identify the presence of His-tagged proteins in samples.
- Optimize protein expression by monitoring the His-tagged proteins level.
- High throughput screening of stable cell lines expressing His-tagged proteins.

II. ASSAY PRINCIPLE

The kit is based on the competitive ELISA method. The His Tag Plate provided is a 384-well microtitre plate that has been pre-coated with a His-tagged protein of 12.7 kDa molecular weight. The immobilized His-tagged proteins compete with the free His-tagged standards(11.3 kDa) or the sample proteins for binding to the biotin-conjugated anti-His antibody. The Streptavidin-HRP is then added to interact with the Biotin conjugated anti-His Antibody. After the washing steps, TMB Solution is added to the plate and color development is stopped by the addition of Stop Solution. Application of the Stop Solution results in a change in color from blue to yellow. The color intensity can be read at 450 nm by a microplate reader. The presence of elevated concentrations of His-tagged protein in the solution results in a reduction in antibody binding to the pre-coated plate. Under optimized test conditions, each absorbance value corresponds to a specific amount of His-tagged protein in the solution. A standard curve is generated using His-tagged protein standards of known concentrations and absorbance values. The concentration of His-tagged protein in unknown samples can be obtained by converting the absorbance in the standard curve.

III. KIT CONTENTS

The kit provides the following reagents and solutions for the measurement of His-tagged protein in biological matrices.

Table 1. Components of the kit

Component	Quantity/Size	Part No.
His Tag Plate (384-well)	1 plate (384 wells, non-removable)	Z1-80

His Tag Standard Stock (10 µg/mL)	1 vial (0.4 mL)	Z1-10
Biotin conjugated Anti-His Antibody	1 bottle (15 mL)	Z1-20
Streptavidin HRP	1 bottle (25 mL)	Z1-30
Sample Dilution Buffer	1 bottle (60 mL)	Z1-60
20× Wash Solution	1 bottle (60 mL)	B-A1-71
Stop Solution	1 bottle (15 mL)	B-A1-51
TMB Solution	1 bottle (25 mL)	B-A1-41
Plate Sealer	2 pieces	N/A

- GenScript offers the 20× Wash Solution (GenScript, B00063) and Sample Dilution Buffer (GenScript, B00062) if the customer requires.

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

- Microplate reader capable of measurement at 450 nm
- Data analysis and graphing software.
- 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 incubator
- Rotary shaker
- Vortex Mixer

VI. PRECAUTIONS

1. Avoid contact with skin, eyes, or clothing with Stop Solution or TMB Substrate. Keep the container tightly closed. In case of an accident, please seek medical advice immediately.

2. Do not use the kit if there is any visible damage to the packaging or kit contents.
3. Do not mix components from different batches. Do not mix with components from other manufacturers.
4. Do not use reagents beyond the stated expiry date.
5. 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 contaminations may occur.
6. Before opening the His Tag Standard Stock (10 µg/mL), quickly span the vial to ensure that all the liquid has collected at the bottom, and prevent the liquid from splashing when opening the lid.
7. Use only distilled or deionized water and clean glassware.
8. 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 own studies when establishing alternate stability criteria that meet their needs.
2. Store specimens at -20°C or lower if not tested immediately. Avoid repeated freeze-thaw cycles.

VIII. PROTOCOL

● Sample Preparation

Minimize the concentration of certain reagents in the sample. For some reagents may interfere with the test result, read the section of **X. ANALYTICAL PERFORMANCE-Reagent Compatibility** carefully.

Samples should not contain any particles/precipitates. Filter the sample or centrifuge as necessary to remove insoluble materials.

For best results, the sample should be adjusted to a neutral pH (6.8-7.4).

● 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 60 mL of 20× Wash Solution with 1140 mL of deionized or distilled water to make 1200 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.

Calibration standards Preparation: Calibration standards should be prepared with a Sample Dilution Buffer to generate eight concentrations: 0, 1, 3, 9, 27, 81, 243, and 729 ng/mL. Preparation of a whole set of standards is recommended in table 2, which is described below as an example.

Table 2. Recommended standard preparation

Standard ID	Dilution Factor	Source	Source Volume (μL)	Sample Dilution Buffer Volume (μL)	Final Volume (μL)	Final Conc. (ng/mL)
AP	13.7	His Tag Standard Stock (10 μg/mL)	72.9	927.1	1000	729
Std1	3	AP	300	600	900	243
Std2	3	Std1	300	600	900	81
Std3	3	Std2	300	600	900	27
Std4	3	Std3	300	600	900	9
Std5	3	Std4	300	600	900	3
Std6	3	Std5	300	600	900	1
BL	/	/	/	600	600	0

Note:

1. The AP (Anchor point) is a selected concentration of His-tagged protein used to improve the fit of the standard curve, and it is not within the quantitative range of the kit.

2. The above calibration standard preparation process is only limited to His Tag Standard Stock (10 μg/mL) provided in this kit. This kit is designed for the semi-quantitative detection of His-tagged proteins in various solutions. For accurate quantification of His-tagged protein expression, it is recommended to use the same protein as the one being tested as a quantitative reference, and to dilute it following the aforementioned method.

- **His Tag Plate (384-well) Preparation**

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

Table 3. Setup of standards and samples on His Tag Plate (384-well)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	BL	BL	S9	S9	S25	S25	S41	S41	S57	S57	S73	S73	S89	S89	S105	S105	S121	S121	S137	S137	S153	S153	S169	S169
B	Std6	Std6	S10	S10	S26	S26	S42	S42	S58	S58	S74	S74	S90	S90	S106	S106	S122	S122	S138	S138	S154	S154	S170	S170
C	Std5	Std5	S11	S11	S27	S27	S43	S43	S59	S59	S75	S75	S91	S91	S107	S107	S123	S123	S139	S139	S155	S155	S171	S171
D	Std4	Std4	S12	S12	S28	S28	S44	S44	S60	S60	S76	S76	S92	S92	S108	S108	S124	S124	S140	S140	S156	S156	S172	S172
E	Std3	Std3	S13	S13	S29	S29	S45	S45	S61	S61	S77	S77	S93	S93	S109	S109	S125	S125	S141	S141	S157	S157	S173	S173

F	Std2	Std2	S14	S14	S30	S30	S46	S46	S62	S62	S78	S78	S94	S94	S110	S110	S126	S126	S142	S142	S158	S158	S174	S174
G	Std1	Std1	S15	S15	S31	S31	S47	S47	S63	S63	S79	S79	S95	S95	S111	S111	S127	S127	S143	S143	S159	S159	S175	S175
H	AP	AP	S16	S16	S32	S32	S48	S48	S64	S64	S80	S80	S96	S96	S112	S112	S128	S128	S144	S144	S160	S160	S176	S176
I	S1	S1	S17	S17	S33	S33	S49	S49	S65	S65	S81	S81	S97	S97	S113	S113	S129	S129	S145	S145	S161	S161	S177	S177
J	S2	S2	S18	S18	S34	S34	S50	S50	S66	S66	S82	S82	S98	S98	S114	S114	S130	S130	S146	S146	S162	S162	S178	S178
K	S3	S3	S19	S19	S35	S35	S51	S51	S67	S67	S83	S83	S99	S99	S115	S115	S131	S131	S147	S147	S163	S163	S179	S179
L	S4	S4	S20	S20	S36	S36	S52	S52	S68	S68	S84	S84	S100	S100	S116	S116	S132	S132	S148	S148	S164	S164	S180	S180
M	S5	S5	S21	S21	S37	S37	S53	S53	S69	S69	S85	S85	S101	S101	S117	S117	S133	S133	S149	S149	S165	S165	S181	S181
N	S6	S6	S22	S22	S38	S38	S54	S54	S70	S70	S86	S86	S102	S102	S118	S118	S134	S134	S150	S150	S166	S166	S182	S182
O	S7	S7	S23	S23	S39	S39	S55	S55	S71	S71	S87	S87	S103	S103	S119	S119	S135	S135	S151	S151	S167	S167	S183	S183
P	S8	S8	S24	S24	S40	S40	S56	S56	S72	S72	S88	S88	S104	S104	S120	S120	S136	S136	S152	S152	S168	S168	S184	S184

Std: Standard number; S: Sample number; BL: Blank Control; AP: Anchor point

Note:

1. His Tag ELISA Detection Kit (384-Well Plate) is non-removable, so we recommend using all the wells at once. If the experimental throughput is relatively low, a detachable 96-well His Tag ELISA Detection Kit (GenScript, L00436) may be a suitable alternative.

2. If not all wells are to be used simultaneously, it is possible to cover the unused wells with plate sealer to prevent contamination. It is imperative that the plate sealer is securely affixed. The sealed plate should be stored at 4°C and clearly labeled to indicate which wells have been utilized and which remain unused. Before reuse, the wells must be inspected for any residual liquid or contamination to ascertain that the remaining wells are uncontaminated. Furthermore, it is advised that the first two columns of wells adjacent to the previously used ones are not employed.

● Test Procedure

His-Tagged Protein and Biotin conjugated Anti-His Antibody Incubation

1. Add 20 µL of the diluted standards, controls and samples to the corresponding wells in the His Tag Plate (384-well).
2. Add 20 µL of Biotin conjugated Anti-His Antibody to all the wells.
3. Cover the plate with a Plate Sealer, mix on a rotary shaker for 30 seconds, and then incubate at 25°C for 30 minutes without shaking.
4. Remove the Plate Sealer. Dump the contents of the wells into waste and wash the plate with 80 µL of 1× Wash Solution five times.
5. Tap the inverted plate onto absorbent paper to remove residual liquid in the wells after the wash steps.

Streptavidin-HRP Incubation

6. Add 40 µL of Streptavidin-HRP to all the wells.
7. Cover the plate with a Plate Sealer and incubate at 25°C for 10 minutes without shaking.

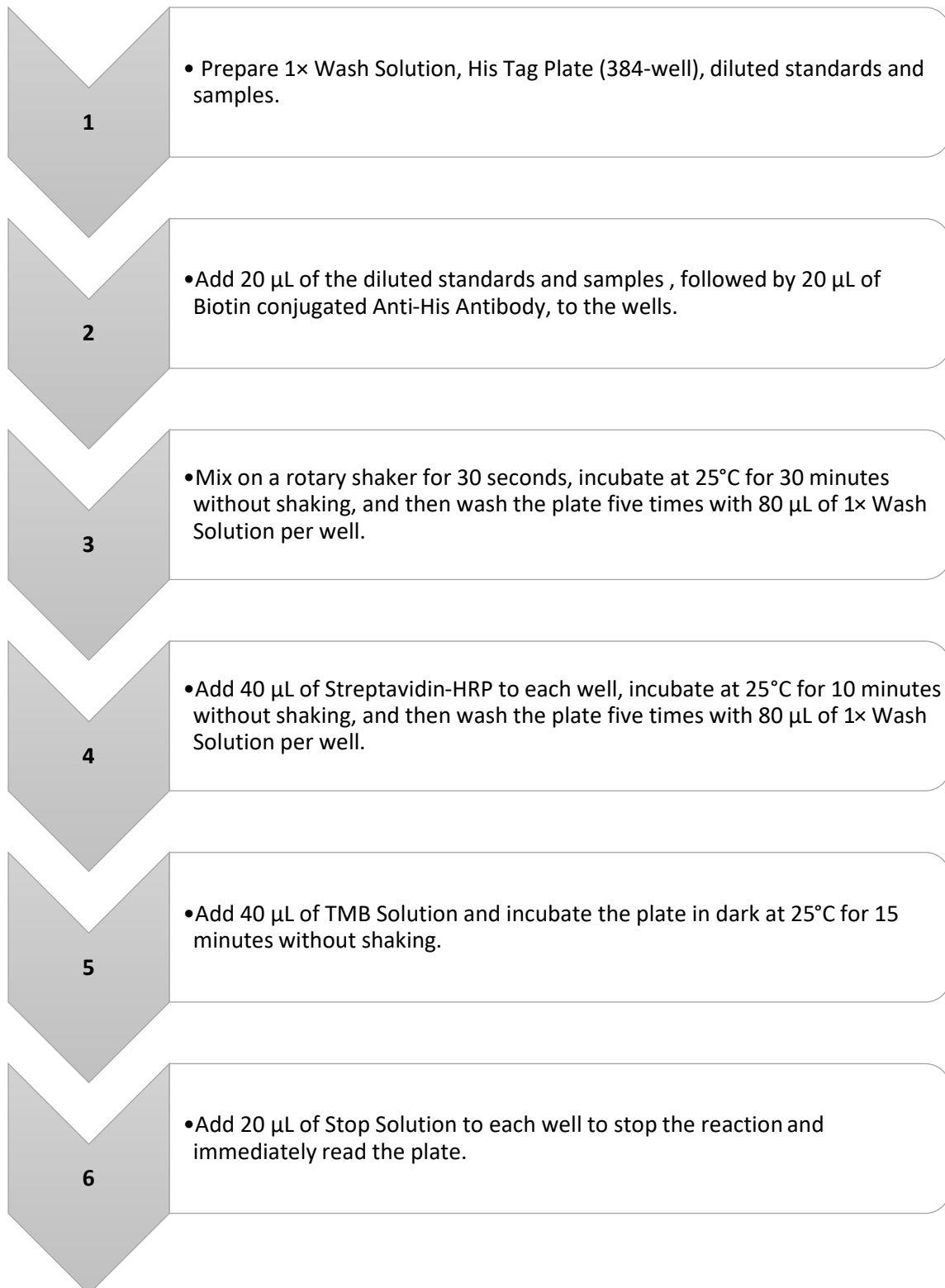
8. Remove the Plate Sealer. Dump the contents of the wells into waste and wash the plate with 80 μ L of 1 \times Wash Solution five times.
9. Tap the inverted plate onto absorbent paper to remove residual liquid in the wells after the wash steps.

Absorbance Measurement and Calculation

10. Add 40 μ L 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).
11. Add 20 μ L of Stop Solution to each well to stop the reaction.
12. Read the absorbance in the microplate reader at 450 nm.
13. Plot the standard curve with the Calibration standards concentration (ng/mL) on the x-axis and the corresponding mean absorbance value on the y-axis.
14. Using a 4-parameter or 5-parameter logistic curve fitting program, calculate the best-fitting linear line ($R^2 > 0.99$) through the points of the standard curve. The amount of His-tagged protein in samples can be determined by extrapolating their OD values to the standard curve.

Note: After adding Stop Solution, the solution in the wells will turn yellow. To ensure the stability of the assay, read the plate at 450 nm immediately after the addition of the Stop Solution. If the sample is diluted, multiply the interpolated value by the dilution factor to calculate the amount of His-tagged protein in the sample.

IX. ASSAY PROCEDURE SUMMARY



X. ANALYTICAL PERFORMANCE

- **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 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 data for His-tagged protein standard curve

His Tag Standard (ng/mL)	Absorbance (OD 450 nm)			Measured His Tag Standard (ng/mL)	CV	Recovery Rate
	Duplicate 1	Duplicate 2	Average			
0	2.6672	2.7283	2.6978	/	/	/
1	2.3134	2.2391	2.2763	1.16	15%	116%
3	1.8494	1.9124	1.8809	2.69	8%	90%
9	1.1055	0.9926	1.0491	9.64	12%	107%
27	0.5196	0.5107	0.5152	26.88	2%	100%
81	0.2486	0.2621	0.2554	70.41	5%	87%
243	0.1241	0.1184	0.1213	272.77	8%	112%
729	0.0863	0.0823	0.0843	/	/	/

Standard Curve for His-tagged protein

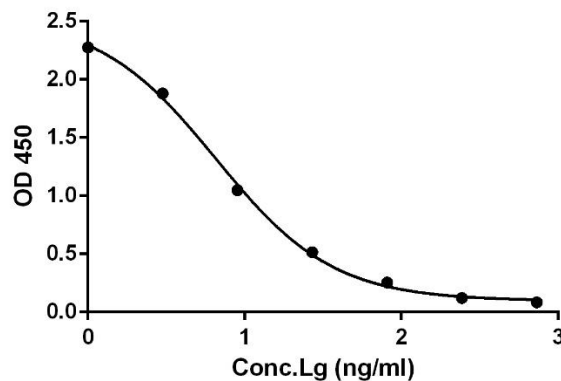


Figure 1: His-tagged protein standard curve

Note: The concentration of 729 ng/mL His-tag standard is the AP (anchor point) used to enhance the fit of the standard curve, and it is not within the quantitative range of the kit.

- **Quantitation Limit and Linear Range**

In accordance with the evaluation method outlined in the <Validation of Analytical Procedures Q2(R2) >, the Quantitation Limit for the His tag standard in this kit is 1 ng/mL, with a linear range of 1 to 243 ng/mL.

Please note that the provided Quantitation Limit and Linear Range apply only to the standards supplied with this kit. Should you intend to use different standards, please re-evaluate in

accordance with the methods specified in the relevant regulations.

- **Measurement Precision**

Intra-assay and inter-assay precision were measured in 3 different concentrations (P1 at 3 ng/mL, P2 at 27 ng/mL, and P3 at 100 ng/mL) of His-tagged protein, using 3 batches of kits.

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

Quality Control	His-tagged protein (ng/mL)	Intra-assay (n=10)			Inter-assay (n=30)		
		Average Measured His-tagged protein (ng/mL)	CV	Recovery Rate	Average Measured His-tagged protein (ng/mL)	CV	Recovery Rate
P1	3	2.83	7.3%	94%	3.02	11.5%	101%
P2	27	27.79	7.4%	103%	27.56	3.6%	102%
P3	100	104.50	9.5%	105%	105.04	5.0%	105%

- **Reagent Compatibility**

The following table lists the interference levels of certain reagents on the results. If the amount of a reagent added exceeds the levels given in the table, it may interfere with the results. We recommend diluting the sample during the experiment to reduce the concentration of the interfering substance below the specified levels. However, due to the variety and complexity of matrices, it is advisable to test the potential effect of the matrix on the experiment before performing it to obtain more accurate results.

Table 3. Reagent compatibility

Reagent	Recommended Use
Triton X-100	≤ 1%
Imidazole	≤ 125 mM
Guanidine HCl	≤ 30 mM
Urea	≤ 0.5 M
SDS	≤ 0.025%
EDTA	≤ 10 mM
β-ME	≤ 160mM
DTT	≤ 10 mM
NaCl	≤ 1 M
CHAPS	≤ 1%
Tween-20	≤ 1%
Glycerol	≤ 1%

XI. 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 prior to the assay.
	Pipette error	Check pipette calibration and repeat assay.
	Components are used from other lots or sources	Never substitute any components from another kit.
	Components are not brought to room temperature prior to the assay	Repeat the assay with components that have been equilibrated to room temperature.
Weak/No Signal	Substrate is not added or added at the wrong time	Follow the manual to add the substrate properly.
	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 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.
	Substrate is contaminated	Use new substrate from the same 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.

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

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