A031-EN.01

resDetect™ GENIUS™ Nuclease ELISA Kit (Residue Testing)

(Enzyme-Linked Immunosorbent Assay)

Pack Size: 96 tests

Catalog Number：CRS-A031

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

INTENDED USE

The GENIUS™ Nuclease ELISA Kit was developed for the detection and quantitative determination of nuclease in samples from downstream processing where nuclease is used as a process or purification aid. It is intended for research use only (RUO).

BACKGROUND

Nucleases are enzymes that degrade nucleic acids, either DNA or RNA. It has been used for the preparation of nuclear extracts by digesting DNA and releasing nuclear proteins intimately associated with DNA. It has also been designed for removing RNA and DNA in biotechnological processing. Thus, the amount of residual nuclease in biological products should be detected and limited.

To support the development of biological products, ACROBiosystems has developed a self-developed universal nuclease quantitative assay kit for the detection and analysis of nuclease residues in biologics through rigorous methodological validation.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of Nuclease by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-Nuclease Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-Nuclease Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of Nuclease bound.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatant samples.
3. Do not use reagents past their expiration date.
4. Do not mix or substitute reagents with those from other kits or other lot number kits.
5. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other

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intermediate dilutions can be in cell culture medium.

6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.

7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
CRS031-C01	Pre-coated Anti-Nuclease Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
CRS031-C02	Nuclease Standard	5 μg	Power	2-8°C	-70°C
CRS031-C03	Biotin-Anti-Nuclease Antibody	20 μg	Power	2-8°C	-70°C
CRS031-C04	Streptavidin-HRP	50 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light
CRS031-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
CRS031-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
CRS031-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
CRS031-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

STORAGE

1. Unopened kit should be stored at 2°C -8°C upon receiving.

2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

Note: *a. Do not use reagents past their expiration date.*

b. Find the expiration date on the outside packaging.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection requirements;

37°C Incubator;

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Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vertexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, the size of the aliquot (CRS031-C02) should not be less than 2 μg, and the size of the aliquot (CRS031-C03) should not be less than 5 μg.

Note: *Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.*

Table 2. Preparation method

ID	Components	Size (96 T)	Storage solution concentration.	Reconstituted water Vol.
CRS031-C02	Nuclease Standard	5 μg	50 μg/mL	100 μL
CRS031-C03	Biotin-Anti-Nuclease Antibody	20 μg	200 μg/mL	100 μL

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

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1.3 Preparation of Biotin-Anti-Nuclease Antibody working fluid:

Dilute Biotin-Anti-Nuclease Antibody to 1.0 μg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

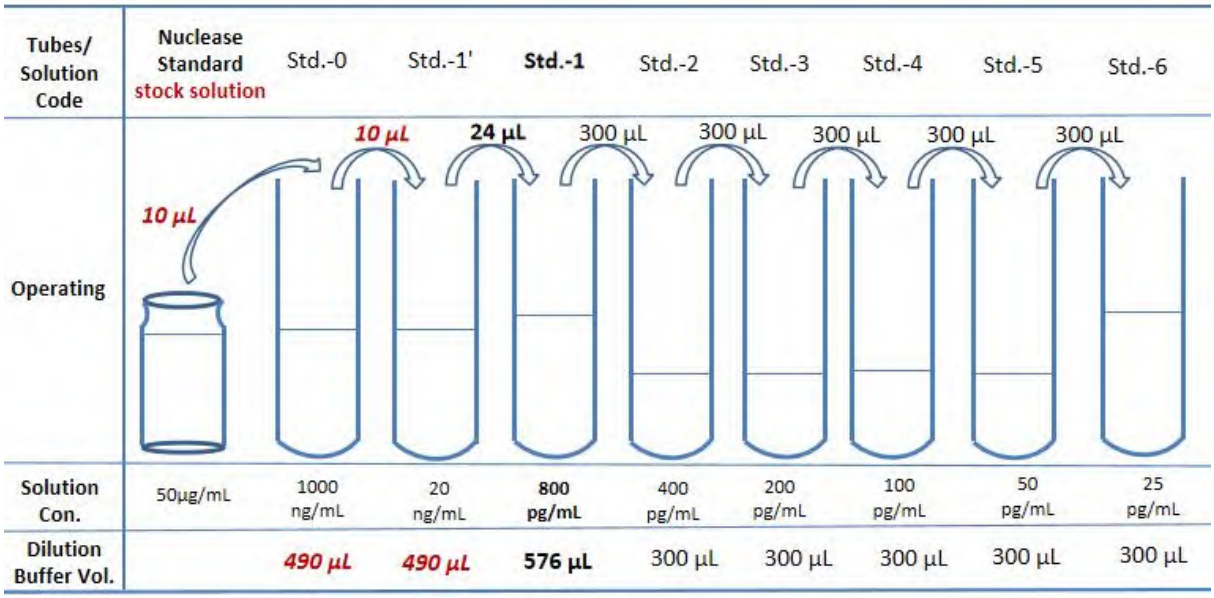
Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

1.5 Sample preparation

If the sample to be tested is the cell supernatant, dilute test sample at 1:2 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:1.

2. Preparation of Standard curve

The concentration of the reconstituted Nuclease Calibrator (CRS031-C02) is 50 μg/mL，prepare (Std.-0) by diluting 10 μL the reconstituted Nuclease Calibrator into 490 μL Sample Dilution Buffer, mix gently well. Then prepare Std.- 1' by diluting 10 μL Std.-0 into 490 μL Sample Dilution Buffer. At last, prepare the highest concentration of standard curve, **Std.-1 (800 pg/mL)**, by diluting 24 μL Std.- 1' into 576 μL Sample Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 μL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.



3. Add Samples

Add 100 μL Calibrator and samples to each well. For blank Control wells, please add 100 μL Dilution Buffer.

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***Note**: It is recommended to set double holes for samples and standard curves to be tested.*

4. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

6. Add Biotin-Anti-Nuclease Antibody

For all wells, add 100 µL Biotin- Anti-Nuclease Antibody (dilute to 1.0 µg/mL) working solution. Please prepare it for one-time use only.

7. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

8. Washing

Repeat step 5.

9. Add Streptavidin-HRP

For all wells, add 100 µL Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

10. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

11. Washing

Repeat step 5.

12. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

13. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

***Note:** The color in the wells should change from blue to yellow.*

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14. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 5 minutes.

***Note**: To reduce the background noise, subtract the value read at OD_{450nm} with the value read at $OD_{630\,nm}$.*

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).

2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

3. Normal range of Standard curve: $R^2 \geq 0.9900$.

4. Detection range: 25 pg/mL-800 pg/mL. If the OD value of the sample to be tested is higher than 800 pg/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 25 pg/mL, the sample should be reported.

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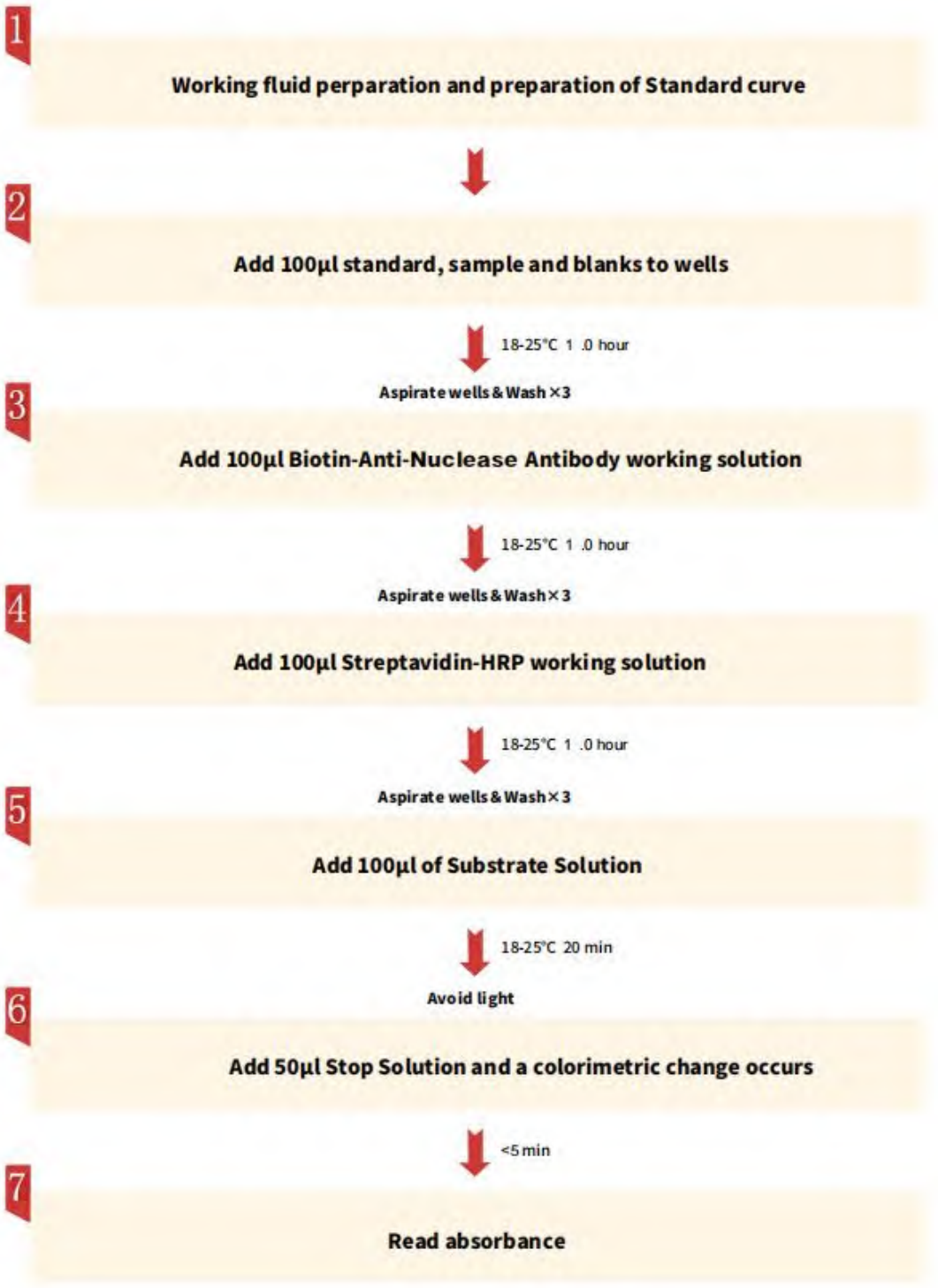
QUICK GUILD



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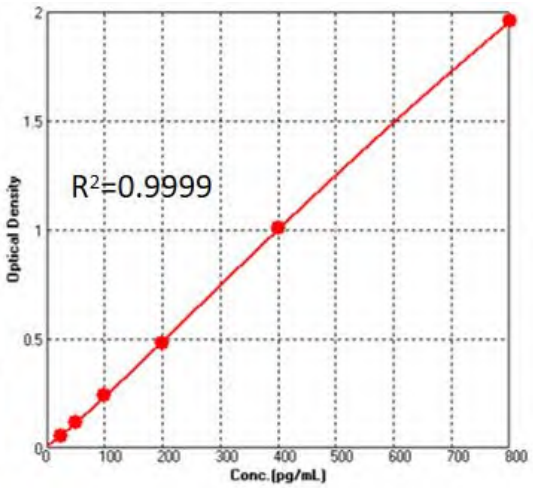
TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (pg/mL)	O.D.-1	O.D.-2	Average	Corrected
800	2.044	2.037	2.041	1.955
400	1.093	1.091	1.092	1.007
200	0.574	0.560	0.567	0.482
100	0.332	0.326	0.329	0.244
50	0.201	0.203	0.202	0.117
25	0.138	0.145	0.142	0.056
0	0.086	0.085	0.086	/



SENSITIVITY

The minimum detectable concentration of Nuclease is 8.254 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision：

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. Inter-assay Precision：

Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (pg/mL)	559.664	133.969	21.916	559.291	136.959	22.597
SD	11.546	6.060	1.552	16.841	5.290	0.590
CV (%)	2.1%	4.5%	7.1%	3.0%	3.9%	2.6%

***Note**: The example data is for reference only.*

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RECOVERY

Three Nuclease with different concentrations were tested to calculate the recovery rate.

Sample(n=3)	Detect Conc.(pg/mL)	Average Detect Conc.(pg/mL)	Average Recovery (%)	Range (%)
High	591.876	574.389	95.7%	90.2-99.3
	550.680			
	596.055			
	541.459			
	591.876			
Middle	283.177	281.890	94.0%	90.7-98.9
	275.739			
	281.825			
	272.017			
	296.691			
Low	53.249	55.805	93.0%	84.7-98.2
	58.943			
	50.821			
	58.475			
	57.536			

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of Nuclease were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (1640)
1:2	Average Recovery (%)	95.6	94.2
	Range (%)	90.2-100.5	89.7-100.2
1:4	Average Recovery (%)	94.5	92.5
	Range (%)	88.7-102.3	89.7-95.7
1:8	Average Recovery (%)	91.0	95.6
	Range (%)	87.4-96.3	93.0-99.4
1:16	Average Recovery (%)	92.5	94.7
	Range (%)	90.9-94.9	87.5-99.1

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***Note**: The example data is for reference only.*

SPECIFICITY

This assay recognizes natural and recombinant Nuclease. No cross-reactivity was observed when this kit was used to analyze the following recombinant factors.

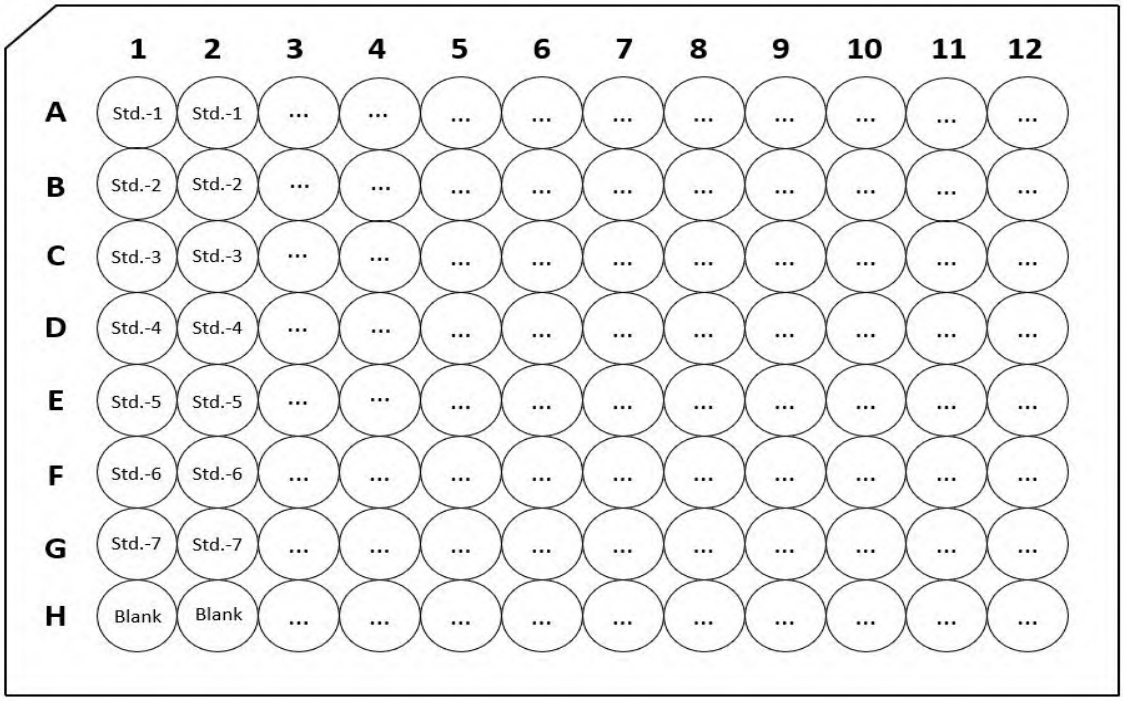
Reactivity	Cap-2'-O-Methyltransferase; T7 RNA Polymerase; CAS9; Cas12a; Pyrophosphatase

INTERFERING SUBSTANCES

Verify potential matrix effects by adding different levels of Cell culture medium、DMSO and HSA to the diluted buffer.

Additive	Tolerated concentration
Cell culture medium (DMEM)	50%
Cell culture medium (1640)	50%
DMSO	2%
HSA	5%

PLATE LAYOUT



***Note:** Blank is a Blank Dilution Buffer hole.*

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TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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