RES96-EN.01

resDetect™ Human Laminin 511 ELISA Kit (Residue Testing)

Catalog Number：RES-A096

Pack Size: 96 tests

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

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

INTENDED USE

The kit is developed for the detection and quantitative determination of Laminin 511 in cell culture supernates. It is intended for research use only (RUO).

BACKGROUND

Laminin 511 E8 is a truncated laminin 511, which is one of the ECM proteins. Laminin E8s serve as functionally minimal forms that retain the full capability for binding to integrins. Laminin 511 E8 consists of the C-terminal end of the alpha 5, beta 1, and gamma 1 chains. The laminin-511 heterotrimer (α5β1γ1) is one of the isoforms to be identified and a potent adhesive and pro-migratory substrate for a variety of normal and tumor cell lines in vitro. Previous studies indicated that Laminin 511 contributes to tumor dissemination and metastasis in advanced breast carcinomas and other tumor types. Additionally, the latest research uncovered that Laminin 511 might be a target antigen and involved in autoimmune pancreatitis. To support the development of cell therapy drugs, ACROBiosystems developed Human Laminin 511 ELISA Kit (Residue Testing) with rigorous methodological validation, which is used for the detection and quantitative determination of Laminin 511 in cell culture supernates. Besides, this kit can also be used for the quantitative determination of GMP human Laminin 511 Protein (ACROBiosystems, cat#GMP-LA1H25) concentrations.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of human Laminin 511 by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-Laminin 511 Antibody. Firstly, add the standard samples provided in the kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-Laminin 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 the 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 human Laminin 511 bound.

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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.
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 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
RES096-C01	Pre-coated Anti-Laminin 511 Antibody Microplate	1 plate	Solid	2-8℃	2-8℃
RES096-C02	Human Laminin 511 E8 Standard	5 μg×2	Powder	2-8℃	-70℃
RES096-C03	Biotin-Anti-Laminin Antibody	20 μg	Powder	2-8℃	-70℃
RES096-C04	Streptavidin-HRP	50 μL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RES096-C05	10×Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
RES096-C06	2×Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
RES096-C07	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RES096-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

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Note: *It is recommended that Streptavidin-HRP be centrifuged briefly before use to deposit liquid from the tube wall or cap to the bottom of the tube.*

SRORAGE

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

2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.

3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

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;

37ºC Incubator;

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

Tubes: 1.5 mL, 10 mL;

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 RES096-C02 should not be less than 2 μg and the size of RES096-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*

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accuracy.

Table 2. Preparation method

ID	Components	Size (96 T)	Storage solution concentration.	Reconstituted water Vol.
RES096-C02	Human Laminin 511 E8 Standard	5 μg	50 μg/mL	100 μL
RES096-C03	Biotin-Anti-Laminin 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.

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

Dilute Biotin-Anti-Laminin Antibody to 0.1 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 1:5 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:4.

2. Preparation of Standard curve

The concentration of the reconstituted human Laminin 511 E8 Calibrator (RES096-C02) is 50 μg/mL，prepare (Std.-0) by diluting 10 μL the reconstituted human Laminin 511 E8 Calibrator into 490 μL Sample Dilution Buffer, mix gently well. Then prepare Std.-1' by diluting 20 μL Std.-0 into 480 μL Sample Dilution Buffer. Finally, prepare the highest concentration of standard curve, Std.-1 (4 ng/mL), by diluting 60 μL Std.-1' into 540 μL Sample Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as

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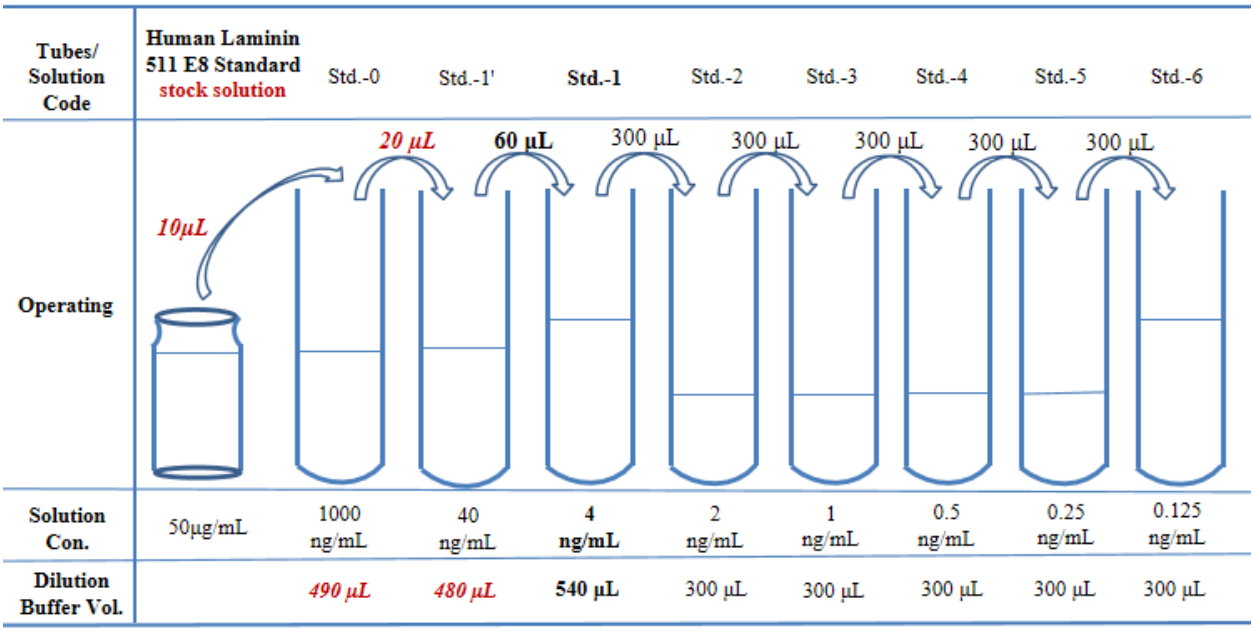
follows: Pipette 300 µL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.



3. Add Samples

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

***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-Laminin Antibody

For all wells, add 100 µL Biotin-Anti-Laminin Antibody (dilute to 0.1 µg/mL) working solution. Please prepare it for one-time use only.

7. Incubation

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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.*

14. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 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 (OD).
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: 0.125 ng/mL-4 ng/mL. If the OD value of the sample to be tested is higher than 4

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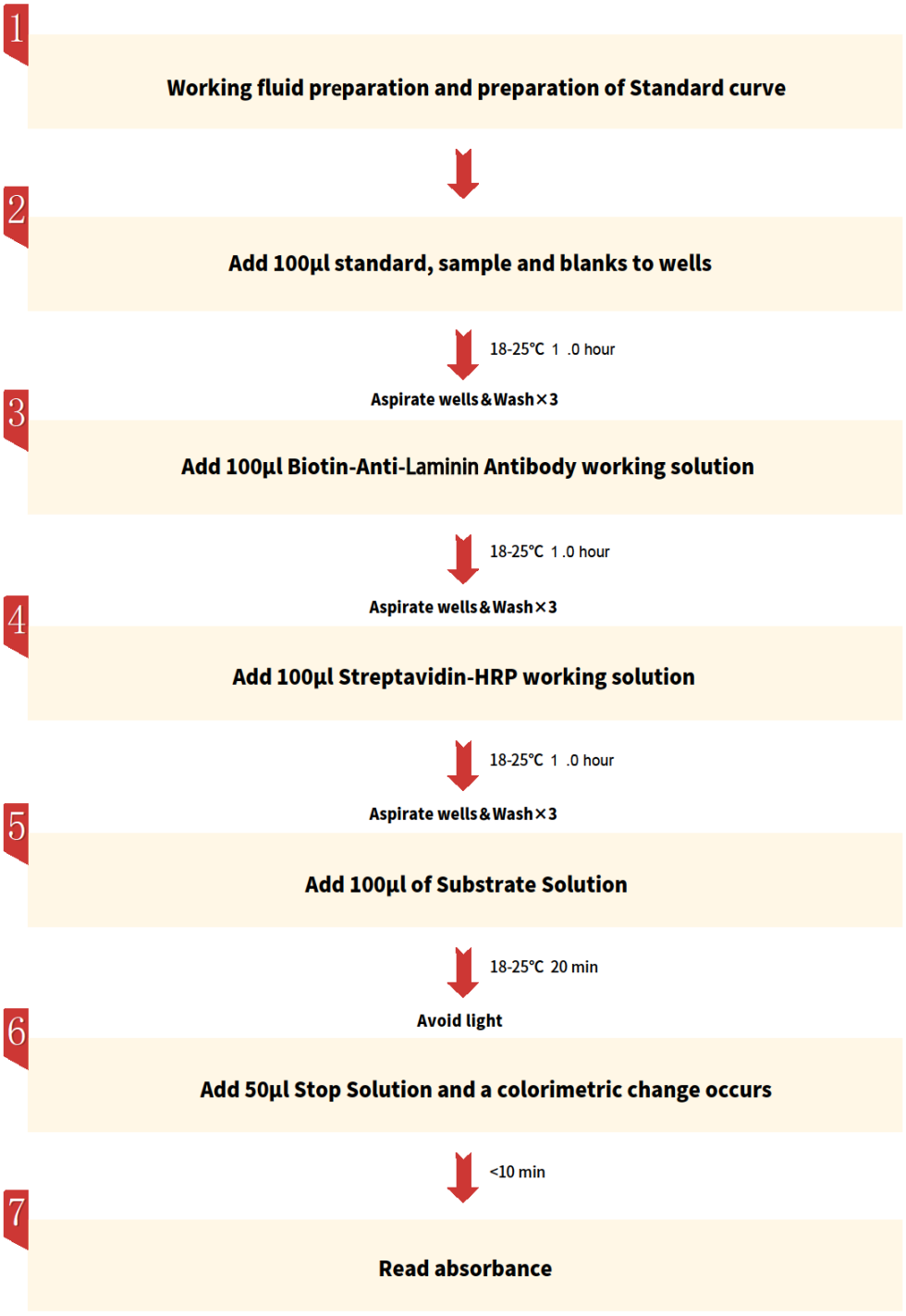
ng/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 0.125 ng/mL, the sample should be reported.

QUICK GUILD



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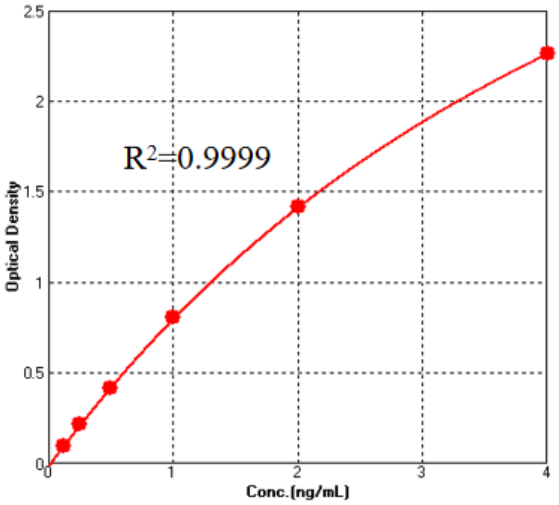
TYPICAL DATA

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 (ng/mL)	O.D.-1	O.D.-2	Average	Corrected
4	2.320	2.343	2.332	2.263
2	1.483	1.482	1.483	1.414
1	0.852	0.891	0.872	0.803
0.5	0.474	0.496	0.485	0.416
0.25	0.284	0.285	0.285	0.216
0.125	0.172	0.162	0.167	0.098
0	0.068	0.070	0.069	/



SENSITIVITY

The minimum detectable concentration of Laminin 511 E8 is 0.068 ng/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 (ng/mL)	2.827	0.710	0.292	2.812	0.707	0.291

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SD	0.113	0.034	0.014	0.020	0.020	0.001
CV (%)	4.0	4.8	4.7	0.7	2.8	0.5

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

RECOVERY

Three Laminin 511 E8 with different concentrations were tested to calculate the recovery rate.

Sample(n=5)	Average% Recovery	Range %
High	93.2	92.6-109.8
Middle	94.8	99.1-103.5
Low	96.9	95.1-109.8

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of Laminin 511 E8 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)	Cell culture medium (mTESR-plus)
1:2	Average Recovery (%)	96.8	93.2	91.7
	Range (%)	82.9-93.2	85.8-93.3	85.2-96.2
1:4	Average Recovery (%)	98.0	100.1	92.8
	Range (%)	85.8-92.7	86.1-90.7	87.1-96.6
1:8	Average Recovery (%)	97.0	96.6	97.5
	Range (%)	87.5-90.7	88.6-92.6	93.9-101.9
1:16	Average Recovery (%)	97.5	101.0	100.2
	Range (%)	84.4-94.9	89.1-98.3	98.2-114.9

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

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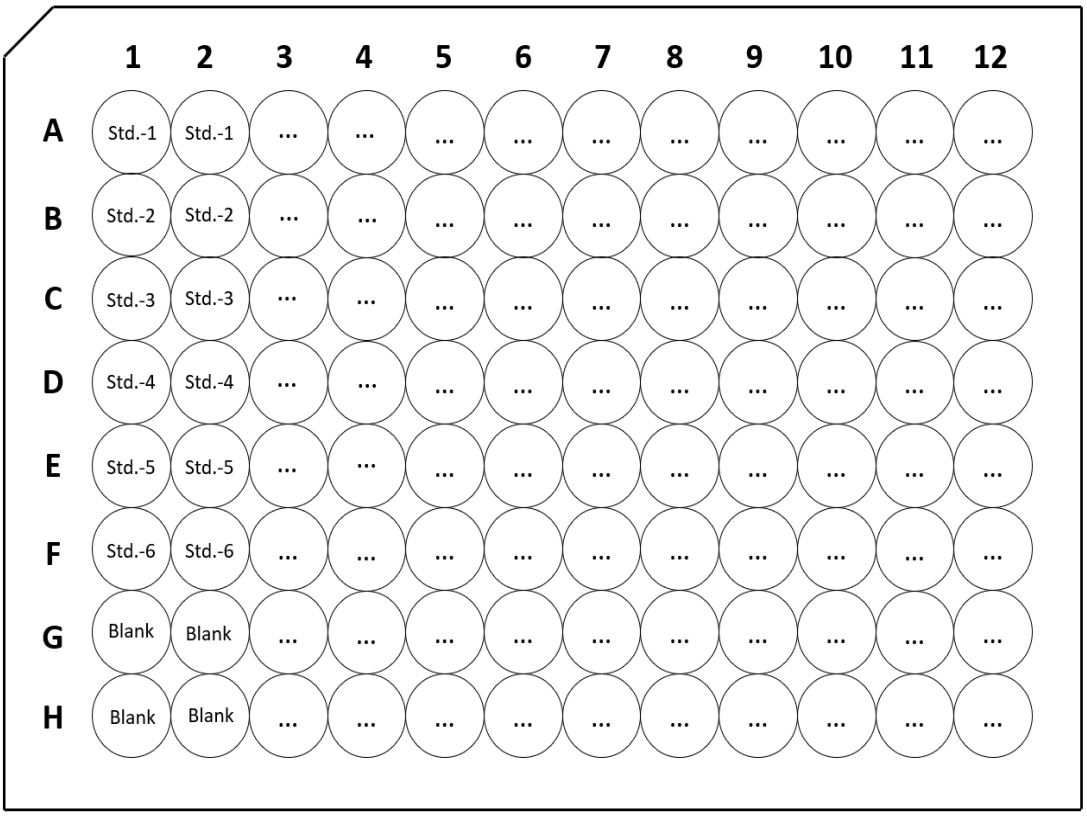
SPECIFICITY

This kit can specifically recognize human Laminin 511. No cross-reactivity was observed when this kit was used to analyze the following recombinant factors.

Human		
Laminin 411	Laminin 211	Laminin 121
Laminin 221	Laminin 111	Laminin 521

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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