RES02-EN.01

resDetect™ Trypsin ELISA Kit

Catalog Number：RES-A002

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 Trypsin. It is used as a tool to aid in optimal purification process development and in routine quality control of in-process streams as well as final product by various biotechnological processes such as cell or tissue culture.

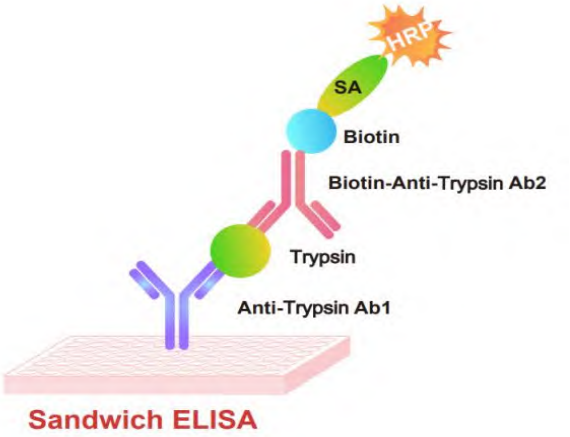
BACKGROUND

Trypsin is an important raw material from the acquisition of early primary cells to the subsequent passage and digestion of adherent cells, and then to large-scale cell factory or microcarrier reactor culture.The amount of Trypsin should be strictly controlled when digesting cells, and the detection of Trypsin residues is also an important link to ensure the normal growth of cells.Because a large amount of Trypsin was used for digestion in the culture stage, it is necessary to test for Trypsin of the downstream part of the production.

Note: *This kit is only suitable for the detection of porcine Trypsin, and Trypsin means porcine Trypsin below.*

PRINCIPLE OF THE ASSAY

This kit uses ELISA sandwich method. The microplates were pre-coated with anti-Trypsin Antibody, the Trypsin in the sample was combined with the fixed anti-Trypsin Antibody on the microplates, and then Biotin-Anti-Trypsin Antibody was added. The antibody-antigen-biotin-labeled antibody complex was formed, and Streptavidin-HRP was finally added to develop color with the substrate, and then terminated with the termination solution. The solution in the plate hole would change from blue to yellow. The absorbance of the sample ($OD_{450\ nm}$ and $OD_{630\ nm}$) was measured with an enzyme-labeled instrument at 450 nm and 630 nm. OD value was positively correlated with Trypsin content in the samples.



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PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Wear appropriate personal protective apparel, please be careful and avoid to contact the reagent with your skin, eyes and clothing. In case of accidental skin exposure, flush with water immediately. Consult a physician if required.
3. Do not use the kit and the all reagents past their expiration date.
4. Do not mix or substitute reagents with those from other kits or other lot number kits.
5. Activity of the conjugate is affected by nucleophiles such as azide, cyanide, and hydroxylamine.
6. If samples generate values higher than the highest standard, dilute the samples with the Dilution Buffer provided in kit and repeat the assay.
7. 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.
8. 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
RES02-C01	Pre-Coated Anti-Trypsin Antibody Microplate	1 plate (8×12 strips)	Solid	2-8°C	2-8°C
RES02-C02	Trypsin Standard (100 ng/mL)	200 µL	Liquid	2-8°C	2-8°C
RES02-C03	Biotin-Anti-Trypsin Antibody	100 µL	Liquid	2-8°C	2-8°C
RES02-C04	Streptavidin-HRP	5 µg	Powder	2-8°C, avoid light	-70°C, avoid light
RES02-C05	1× Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES02-C06	20×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES02-C07	Substrate Solution	12 mL	Liquid	2-8°C	2-8°C
RES02-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

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STORAGE

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;

Orbital microtiter plate shaker：For shaking the plate in immunological steps;

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

1. Take out the kit, equilibrate all reagents and samples to room temperature (20℃-25℃) before use, check that each buffer and standard solution are clear and transparent, make sure these solution are evenly mixed.

2. Reconstitute the provided lyophilized materials to stock solutions with sterile deionized water as recommended in the following table, Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vortex. The reconstituted stock solutions should be stored at -70℃. Avoid freeze-thaw cycles. It is recommended that the number of freezing and thawing should not exceed 1 time.

Note: *Streptavidin-HRP stock solution should be protected from light.*

Table 2. Preparation method

ID	Components	Size (96 T)	Storage solution concentration.	Reconstituted water Vol.
RES002 -C04	Streptavidin-HRP	5 µg	100 µg/mL	100 µL

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RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 25 mL 20×Washing Buffer with ultrapure water/deionized water to 500 mL. Please prepare it for one-time use only.

1.2 Preparation of Biotin-Anti-Trypsin Antibody:

Biotin-Anti-Trypsin Antibody is diluted to a factor of 100 with 1× Dilution Buffer (RES002-C05) according to the experimental dosage (50μL/well). This working solution should be used immediately. Refer to Table 3 for configuration methods:

Table 3. Preparation method

Tests	Working solution	Biotin-Anti-Trypsin Antibody	1×Dilution Buffer
96Tests	6000 μL	60 μL	5940 μL

1.3 Preparation Of Streptavidin-Hrp Working Fluid:

The reconstructed Streptavidin-HRP storage solution is diluted to 0.1μg/mL by 1×Dilution Buffer (RES02-C04) according to the experimental dosage (100 μL/well). The prepared working fluid should avoid light.Please prepare it for one-time use only. Refer to Table 4 for configuration methods:

Table 4. Preparation method

Tests	Working solution	Streptavidin-HRP	1×Dilution Buffer
96Tests	11000 μL	22 μL	10978 μL

2. Preparation of Standard Curve

This kit can be used for the quantitative detection of Trypsin. The kit contains the Trypsin Standard (100 ng/mL) standard for establishing the standard curve.

Note: *Diluted standards should be used within 30 minutes of preparation.*

In order to counteract any standard sticking, we recommend changing tips between each dilution.

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The recommended Trypsin standard dilution procedure is listed and illustrated below:

2.1 Bring the Trypsin standard stock solution to room temperature, the original concentration is 100 ng/mL.

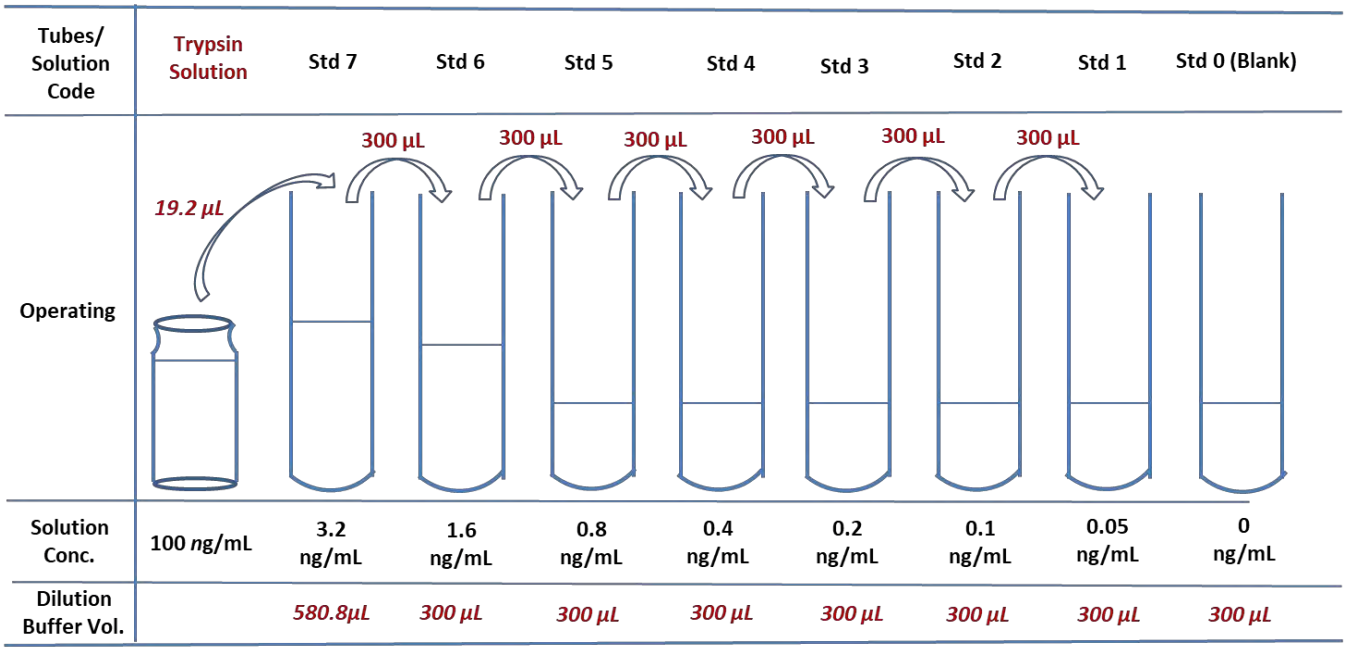
2.2 Dilute the 100 ng/mL of standard stock solution 31.25 fold with 1×Dilution Buffer to 3.2 ng/mL (Std 7: 3.2 ng/mL).

2.3 The standard curve is prepared by 2 times gradient dilution at the highest concentration point of the standard curve (Std 7:3.2 ng/mL), as shown below (taking the dilution volume of each concentration point of the standard product as 600 µL). After each step of dilution, the remaining volume of the standard product should not be less than 0.1 mL;

- Add 300 µL of 1× Dilution Buffer to each Std 6 to Std 1 tube;

-Add 300 µL Std 7 to 300µL 1×Dilution Buffer, mix gently and repeat the serial dilution to make 6 Trypsin standard solutions: Std 6, Std 5, Std 4, Std 3, Std 2, Std 1.

- Std 0 (Blank) is 1×Dilution Buffer alone.



3. Add the Sample and Biotin-Anti-Trypsin Antibody Working Solution

The sample to be tested and a series of diluted standards were added to the reaction well with 50 µL per well and then immediately with 50 µL Biotin-Anti-Trypsin Antibody.

Note: *Multiple holes are recommended for samples to be tested and standard curves.*

4. Incubation

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Seal the plate with microplate sealing film and incubate at room temperature (20℃-25℃) for 1 hour on orbital shaker at 400-600 rpm.

5. Washing

Thorough washing is essential to proper performance of this assay. Automated plate washing systems or manual wash procedure be selected according to your own experimental conditions.

Remove the remaining solution of the wells, wash the wells by add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 min, remove any remaining 1×Washing Buffer by aspirating or decanting, invert the plate and blot it against lint free paper towels to remove any remaining wash buffer. Please note that the complete removal of the washing buffer is essential.

Repeat the wash step above for 3 times.

6. Add Streptavidin-HRP Solution

Each well requires 100 µL of Streptavidin-HRP working solution.

7. Incubation

Seal the plate with microplate sealing film and incubate at room temperature (20℃-25℃) for 30 min on orbital shaker at 400-600 rpm.

8. Washing

Repeat step 5.

9. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (20℃-25℃) for 20 min, avoid light. Do not shake.

10. 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.*

11. Data Recording

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

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***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。

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. The concentration is calculated by multiplying it by the corresponding dilution.

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

4. Detection range: 0.05 ng/mL - 3.2 ng/mL. If the OD value of the sample to be tested is higher than the highest standard (3.2 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 0.05 ng/mL, the sample residual should be reported＜0.05 ng/mL.

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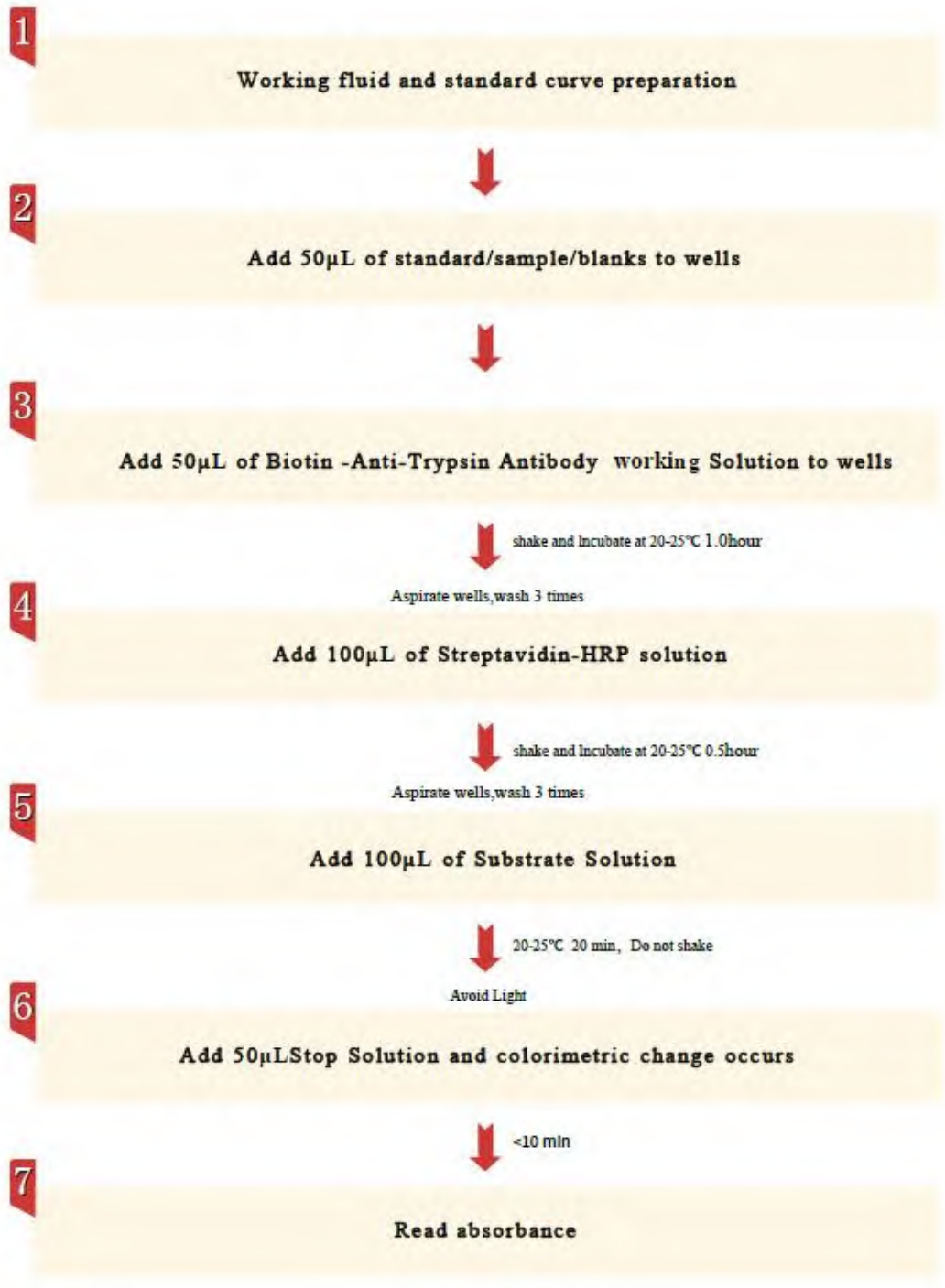
QUICK GUILD



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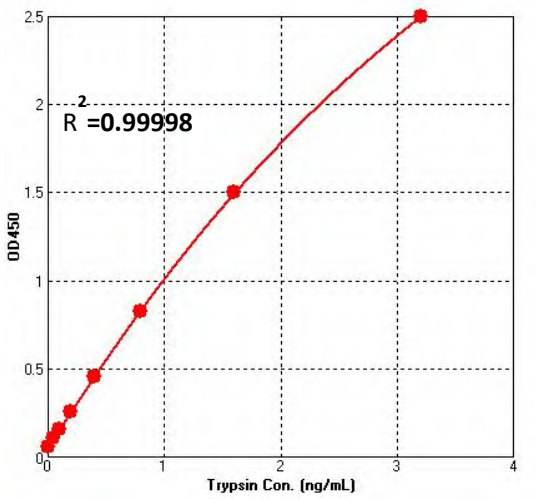
TYPICAL DATA

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.

Standard Num.	Concentration	OD_{450nm}
Standard 7	3.2 ng/mL	2.280
Standard 6	1.6 ng/mL	1.337
Standard 5	0.8 ng/mL	0.742
Standard 4	0.4 ng/mL	0.431
Standard 3	0.2 ng/mL	0.247
Standard 2	0.1ng/mL	0.156
Standard 1	0.05ng/mL	0.105
Standard 0	0 ng/mL	0.058



SENSITIVITY

The minimum detectable concentration of resDetect™ Trypsin ELISA Kit is 0.003801 ng/mL

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision. Intra-Assay Precision CV≤15%.

2. Inter-assay Precision

Three samples of known concentration were tested in ten separate assays to assess inter-assay precision. Inter-Assay Precision CV≤15%.

	Intra-assay Precision			Inter-assay Precision		
Sample	3.2	0.5	0.05	3.2	0.5	0.05
n	10	10	10	10	10	10
Mean (ng/mL)	3	0.49	0.06	3.19	0.4	0.05
SD	0.095	0.019	0.002	0.017	0.008	0.003

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CV (%)	4	3	2	0.054	1.96	4.95

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

ACCUEACY

Five samples of known concentration were tested ten times on one plate to assess Accuracy， Accuracy recovery rate 80%-120%.

Sample Conc.(ng/mL)	3.2	2.5	0.5	0.1	0.05
n	10	10	10	10	10
Mean (ng/mL)	2.392	2.064	0.541	0.165	0.117
SD	0.095	0.043	0.019	0.002	0.002
CV (%)	3.98	2.09	3.48	1.41	1.81
Recovery（%）	94	97	97	106	113

SPECIFICITY

Specificity-1: High, medium and low concentrations of Trypsin were added to MDCK Cell, HEK293 Cell, CHO Cell and Vero Cell, the recovery rate of Trypsin was used as the specific validation index. Recovery rate 75%-120%.

Sample	MDCK			HEK293			CHO			Vero		
Cells.Con.（cells/mL）	2.8×10^6			4×10^6			2×10^6			3.6×10^6		
Dilution Factor	2			2			2			2		
Added Trypsin Conc.(ng/mL)	3.2	0.5	0	3.2	0.5	0	3.2	0.5	0	3.2	0.5	0
Mean (ng/mL)	3.25	0.55	0	2.86	0.47	0	2.82	0.38	0	2.74	0.41	0
Recovery（%）	102	110	/	89	85	/	88	77	/	86	81	/

Specificity-2: It was proved that there was no cross with bovine trypsin.

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MATRIX INTERFERENCE

We have conducted interference effect test about frequently-used buffers, adding the known concentration of Trypsin standard into the buffer, and the calculated recovery rate was 80%-120%. they have excellent buffer compatibility. For specific buffers, it is recommended that you verify recovery to determine the optimal dilution ratio.

	Trypsin	
Matrix	Recovery（%）	Dilution Factor
20 mM L-histidine with 0.1% (w/v) PF68, pH6.0	101	2
20 mM L-histidine with 0.4% (w/v) Tween-80, pH6.0	96	2
1×PBS, pH7.3	81	2
1×PBS, pH7.3 with 11% Trehalose	83	2
20 mM L-histidine, pH6.0	96	2
50 mM Tris,100mM Glycine, pH7.5	92	2
100 mM Tris,20mM Sodium citrate, pH7.5	89	2
20 mM L-histidine 10% trehalose,pH6.0	89	2
50 mM Na Acetate, pH 3.5	91	2
25 mM Phosphate, pH 7.5	83	4
100 mM Glycine, pH 3.5	92	2
100 mM Triscitrate, 7.5	95	2

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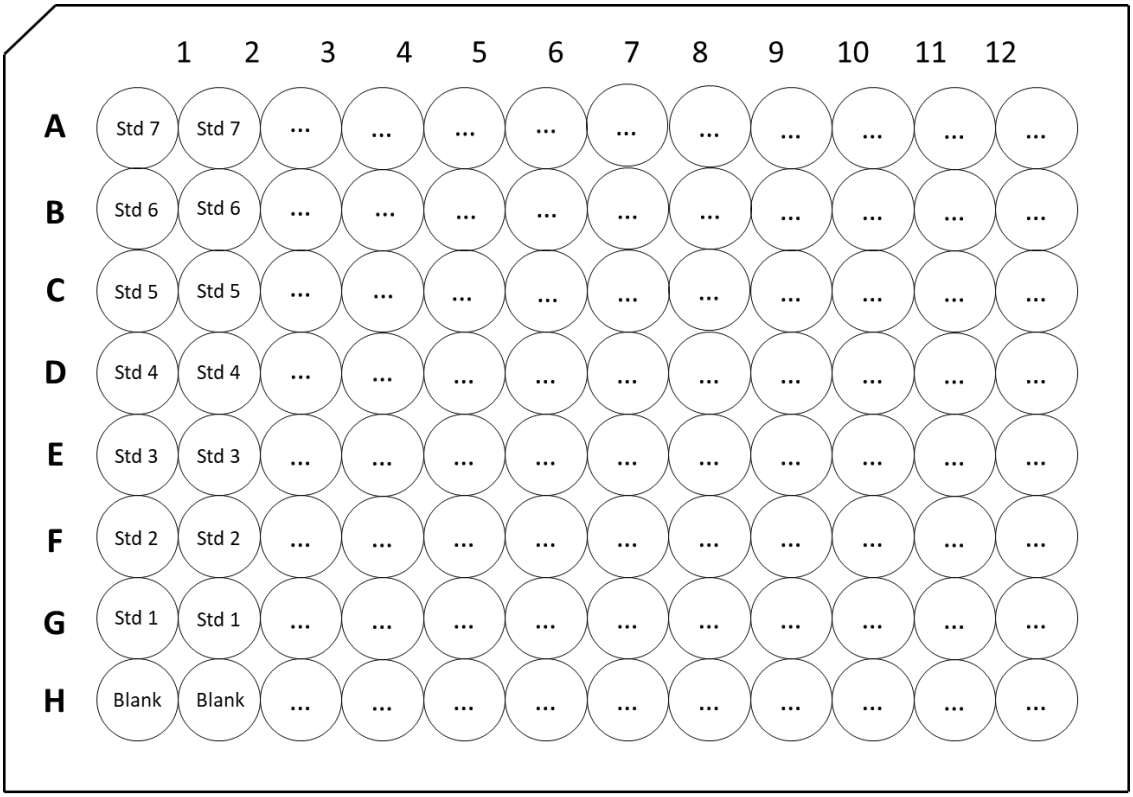
LATE LAYOUT



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

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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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