# Human IL-2 HP ELISA Kit

Catalog NO.: RK04741HP

version: 20230828

This package insert must be read in its entirety before using this product

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## Background

IL-2, or interleukin-2 (interleukin 2), also known as T cell growth factor (TCGF), is a glycosylated α-helical polypeptide with a molecular weight of approximately 15-18 kDa that belongs to the γc cytokine family and exists as a monomer with an extremely short half-life (<30 min). Mature human IL-2 is composed of 133 amino acids remaining from a signal peptide of 153 amino acids clipped by 20 amino acids. The mature structural domain has an α-helical structure in its natural state, and post-translational processing includes O-glycosylation of the third Thr site and disulfide bond formation. There are three cysteine residues (Cys) in the protein chain, located at positions 58, 105 and 125. The formation of disulfide bonds by the binding of cysteine residues at positions 58 and 105 is a necessary prerequisite for the protein to be active. Mature human IL-2 has 73%, 66%, 78% and 97% amino acid homology with canine, rat, cat and monkey IL-2, respectively. Although human IL-2 has only 60% amino acid homology with mouse IL-2, human IL-2 activates IL-2 receptors on mouse cells. γδ T cells, activated CD4+ and CD8+ T cells, neuronal cells, microglia, and hematopoietic stem cells are capable of secreting IL-2.

The IL-2 receptor (IL-2R) is a heterotrimer consisting of a 55 kDa CD25/IL-2 Rα chain, a 70 kDa IL-2 Rβ and a 65 kDa γc chain. IL-2 first binds to IL-2 Rα to form a dimeric complex and then recruits IL-2 Rβ and γc to form a tetrameric signaling complex. In addition to IL-2, IL-2 Rβ is also a component of the IL-15 tetrameric signaling complex, while γc is a common chain shared by IL-4, IL-7, IL-9, IL-15 and IL-21 receptors. IL-2 plays an important role in the cell proliferation of T cells, natural killer cells and B cells in different response phases induced by antibody stimulation. in addition, IL-2 regulates the expression of gamma interferon, major histocompatibility antigens, stimulates the proliferation and differentiation of activated B cells, increases the activity of natural killer cells and inhibits the proliferation of granulocytes/macrophages. IL-2 also induces the proliferation and differentiation of oligodendrocytes IL-2 also induces the proliferation and differentiation of oligodendrocytes. IL-2 binds to its receptor and activates the JAK-STAT signaling pathway through phosphorylation, which transmits the signal to the nucleus for regulation of target genes. In addition, IL-2 can also activate the downstream Ras/Raf/MEK/MAPK and PI3K/Akt/P70S6K signaling pathways

by forming Grb2/SOS complexes under the action of Shc protein.

IL-2 is mainly used clinically for the treatment and adjuvant therapy of various malignancies and infectious diseases because of its immune enhancer, antitumor and anti-infective properties. Initial efficacy has also been observed in the treatment of transplant rejection and autoimmune diseases with monoclonal antibodies to IL-2 or its receptor. New indications for IL-2 have also been gradually developed, allowing for new clinical applications.

## Introduction

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of IL-2 in human serum, plasma, cell lysates, cell culture.

| Features | Specifications |
|---|---|
| Sensitivity | 0.078 pg/mL |
| Detection Range | 1.56-100 pg/mL |
| Suitable Sample | serum, plasma, cell lysates, cell culture |
| Specificity | No significant cross-reactivity of similar compounds was found (see Specificity) |
| Recommended dilution | See sample preparation section below for details |

## Principle of the Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. An monoclonal antibody specific for human IL-2 has been pre-coated onto a microplate.Standards and samples are pipetted into the wells and any IL-2 present is bound by the immobilized antibody. After washing away any unbound substances, and then a detection antibody specific for IL-2 is added to the wells and binds to the combination of capture antibody IL-2 in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate solution is added to the wells and color develops in proportion to the amount of IL-2 bound in the initial step. The color development is stopped and the absorbance is measured.

## Material Provided & Storage Conditions

Unopened kits can be stored at 2-8°C for 1 year, and opened products must be used within 1 month.

| Part | Size | Cat. No. | Storage of opened/reconstituted material |
|---|---|---|---|
| Human IL-2 Microwell Plate Coated | 8×12 | RM96676 | Put the unused slats back in the aluminum foil bag with the desiccant and reseal them. They can be stored at 2-8°C for 1 month. |
| Human IL-2 Standard Lyophilized | 50pg*4vial | RM96677 | It is not recommended to use again after redissolving. |
| Human IL-2 Concentrated Biotin Conjugate Antibody (100×) | 1 ×120ul | RM96678 | Store at 2-8°c for 1 month * |
| Streptavidin-HRP Concentrated (100×) | 1 ×120ul | RM96679 | Store at 2-8°c for 1 month * |

| | | | |
|---|---|---|---|
| Quality Control | 3.125pg*2 vials | RM96677C | It is not recommended to use again after redissolving. |
| Standard/Sample Diluent (R1) | 1 ×20mL | RM00023 | Store at 2-8℃ for 1 month * |
| Biotin-Conjugate Antibody Diluent (R2) | 1 ×12mL | RM00024 | |
| Streptavidin-HRP Diluent(R3) | 1 ×12mL | RM00025 | |
| Wash Buffer(20x) | 1 ×30mL | RM00026 | |
| TMB Substrate | 1 ×12mL | RM00027 | |
| Stop Solution | 1 ×6mL | RM00028 | |
| Plate Sealers | 4 Strips | | |
| Specification | 1 | | |

## Other Supplies Required

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 570 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. Incubator.
6. Test tubes for dilution of standards and samples.

## Precautions

1. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
2. Variations in sample collection, processing, and storage may cause sample value differences.
3. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
4. Stop Solution contains strong acid. Wear eye, hand, and face protection.
5. Please perform simple centrifugation to collect the liquid before use.
6. Do not mix or substitute reagents with those from other lots or other sources.
7. Adequate mixing is particularly important for good result. Use a mini-vortexer at the lowest frequency.
8. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all diluents.
9. Both the sample and standard should be assayed in duplicate, and reagents should be added in sequence in accordance with the requirement of the specification.
10. Reuse of dissolved standard is not recommended.

11. The kit should not be used beyond the expiration date on the kit label.
12. The kit should be away from light when it is stored or incubated.
13. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma, and other biological fluids in accordance with NCCLS regulations.
14. To avoid cross contamination, please use disposable pipette tips.
15. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
16. This assay is designed to eliminate interference by other factors present in biological samples.
17. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.
18. The 48T kit is also suitable for the specification.

## Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Cell Culture Supernatant**: Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Serum**: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Plasma**: Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)

**Cell Lysates :** Cells need to be lysed before assaying according to the following directions.Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000 × g for 5 minutes (suspension cells can be collected by centrifugation directly).

Wash cells three times in cold PBS.Resuspend cells in fresh lysis buffer with concentration of $10^7$ cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.Centrifuge at 1,500 × g for 10 minutes at 2-8 ℃ to remove cellular debris. Assay immediately or aliquot and store at ≤-20 ℃.

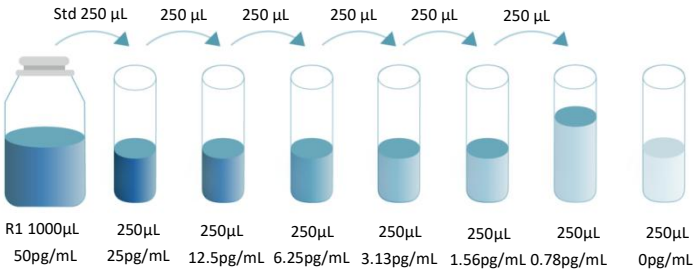
**Note**: It is suggested that all samples in one experiment be collected at the same time of the day. Avoid hemolytic and hyperlipidemia sample for serum and plasma.

## Reagent Preparation

Bring all reagents to room temperature before use. If crystals have formed in the Wash buffer（20x）, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.

**Standard** - Reconstitute the Standard Lyophilized with 1.0mL Standard/Sample Diluent(R1). This reconstitution produces a stock solution of 50pg/mL. Mix gently until completely dissolved and allow the lyophilisate to dissolve to a concentration of 50pg/mL prior to making dilutions.

Use the 100pg/mL standard stock to produce a dilution series (below) with Standard/Sample Diluent(R1). Mix each tube thoroughly and change pipette tips between each transfer (recommended concentration for standard curve: 50, 25, 12.5, 6.25, 3.13,1.56,0.78,0pg/mL). Use diluted standards within 60 minutes of preparation.




**Human IL-2 Control** - Reconstitute the Standard Lyophilized with 1.0mL Standard/Sample Diluent(R1),Mix gently until completely dissolved .

**Working Biotin Conjugate Antibody** - Dilute 1:100 of Concentrated Biotin Conjugate Antibody (100x) with Biotin-Conjugate Antibody Diluent (R2) before use, for example: Add 20 µL of Concentrated Biotin Conjugate Antibody (100x) to 1980 µ L Biotin-Conjugate Antibody Diluent (R2) to prepare 2000 µL Working Biotin Conjugate Antibody Buffer.

**Working Streptavidin-HRP** - Dilute 1:100 of Concentrated Streptavidin-HRP (100x) with Streptavidin-HRP Diluent (R3) before use, for example: Add 20 µ L of Concentrated Streptavidin-HRP (100x) to 1980µL Streptavidin-HRP Diluent (R3)

to prepare 2000μL Working Streptavidin-HRP Buffer.

**1 x Wash Buffer** - If crystals have formed in the Wash buffer （20x）, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 1:20 with double distilled or deionized water before use, for example : Add 20 mL of Wash Buffer Concentrate to 380 mL of deionized or distilled water to prepare 400 mL of Wash Buffer.

## Sample preparation

For different samples, the appropriate dilution level should be chosen on a case-by-case basis.

1. Cell supernatant: As cell supernatant samples vary considerably depending on the experimental conditions, it is recommended to carry out a pre-test to determine the appropriate dilution.

2.Serum: Samples are tested and 2-fold dilution of normal serum/plasma samples is recommended to eliminate

quantitative inaccuracies caused by sample matrix interference. Samples containing more than 50 pg/mL of human IL-2 should be diluted 2-fold or more with Standard/Sample Diluent (R1) or PBS until the diluted sample falls within the detection range of the kit.

**Dilution Method**

For 10 fold diluition: One-step dilution. Add 50 µL sample to 450 µL sample diluent to yield 100 fold dilution. .

For 100 fold dilution: One-step dilution. Add 5 µL sample to 495 µL sample diluent to yield 100 fold dilution.

For 1000 fold dilution: Two-step dilution. Add 5 µL sample to 95 µL sample diluent to yield 20 fold dilution, then add 5 µL 20 fold diluted sample to 245 µL sample diluent, after this, the neat sample has been diluted at 1000 fold successfully.

Each dilution step should be performed at a minimum of 3 µL and at a maximum of 100-fold dilution. Each dilution step should be mixed well to avoid foaming.

## Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal properly.
3. Add 100 µL Standard/sample Diluent (R1) in a blank well.
4. Add 100 µL different concentration of standard、Control or sample in other wells, Cover with the adhesive strip provided. Incubate for 2 hours at 37℃. record the plate layout of standards and sample assay.
5. Prepare the Concentrated Biotin Conjugate Antibody (100x) Working Solution 15 minutes early before use.
6. Add wash buffer 350 µL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.Pat the plate on paper towel to remove residual liquid in the wells.
7. Add 100 µL Working Biotin Conjugate Antibody in each well, cover with new adhesive Sealer provided.Incubate

for 1 hour at 37℃.

8. Prepare the Streptavidin-HRP Concentrated (100x) Working Solution 15minutes early before use.
9. Repeat the aspiration/wash as in step 6.
10. Add 100 μL Working Streptavidin-HRP in each well, cover with new adhesive Sealer provided.Incubate for 0.5 hour at 37℃.
11. Repeat the aspiration/wash as in step 6.
12. During the incubation, turn on the microplate reader to warm up for 30 minutes before measuring.
13. Add 100 μL TMB Substrate to each well. Incubate for 15 minutes at 37℃ .Protect from light.
14. Add 50 μL Stop Solution, determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. This subtraction will correct for optical imperfections in the plate.

## Assay Procedure Summary

Prepare the standard and reagents

↓

Add 100ul of standards or test samples to each well

Incubate for 2 hours at 37℃, then wash 3 times,

Completely pat dry after plate washing

↓

Add 100ul Working Biotin Conjugate Antibody

Incubate for 1 hour at 37℃, then wash 3 times,

Completely pat dry after plate washing

↓

Add 100ul Working Streptavidin-HRP

Incubate for 0.5 hour at 37℃, then wash 3 times,

Completely pat dry after plate washing

↓

Add 100ul Substrate Solution

Incubate for 15-20 min at 37℃ under dark condition

↓

Add 50ul Stop Solution

↓

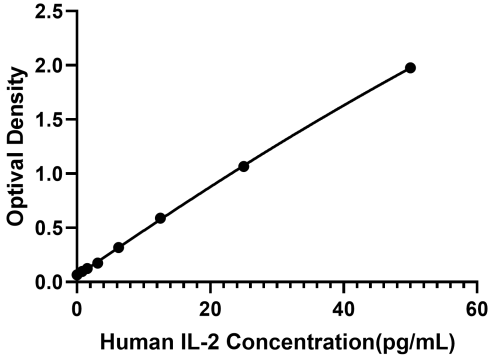
Detect the optical density within 5 minutes under 450nm.

Correction Wavelength set at 570nm or 630nm

## Calculation of Results

1. Average the duplicate readings for each standard, control and sample, and the OD for each test should be subtracted from the OD for the sub-wavelengths.
2. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the IL-2 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. The sample read-outs from the curve must be multiplied by the dilution factor of the sample in order to obtain the concentration in the original sample.
4. Samples that fall above this interval should be diluted accordingly and re-analyzed. Samples that fall below this interval are too low to be accurately quantified by this method.

## Typical Data




| pg/mL | Repeat 1<br>OD450-OD630 | Repeat 2<br>OD450-OD630 | AVE |
|---|---|---|---|
| 0 | 0.0947 | 0.0931 | 0.0939 |
| 0.78125 | 0.1356 | 0.1739 | 0.15475 |
| 1.5625 | 0.1742 | 0.1694 | 0.1718 |
| 3.125 | 0.2465 | 0.2435 | 0.245 |
| 6.25 | 0.3862 | 0.3911 | 0.38865 |
| 12.5 | 0.6394 | 0.6842 | 0.6618 |
| 25 | 1.1686 | 1.2645 | 1.21655 |
| 50 | 2.0906 | 2.1675 | 2.12905 |

The standard curves are provided for demonstration only. The concentration calculations should be based on difference data

## Sensitivity

The minimum detectable dose (MDD) of IL-2 typically less than 0.078pg/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## Specificity

This assay recognizes both recombinant and natural human IL-2. The factors listed below were prepared at 2ng/mL and assayed for cross-reactivity. No significant cross-reactivity was observed with the following:

| Recombinant human | Recombinant mouse | Recombinant rat |
|---|---|---|
| G-CSF | IL-6 | IL-2 |
| IL-2R | IL-4 | |
| IL-8 | IL-5 | |
| GM-CSF | IL-2 | |
| IL-3 | IL-1B | |
| TGF-beta | | |
| IL-1B | | |
| IL-4 | | |
| TNF-a | | |
| IL-1α | | |
| IL-6 | | |

**Note:**

Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between IL-2 and all the analogues, therefore, cross reaction may still exist.

## Precision

Intra-plate Precision

Samples containing 5 different concentrations of the target were tested with the same kit, and each sample was analysed in the same replicate 3 times for 2 days.

Three batches of kits were sampled with an Intra-Assay: CV<10%

| Lot 1 | LLOQ | LQC | MQC | HQC | ULOQ |
|---|---|---|---|---|---|
| Number of Replicates | 6 | 6 | 6 | 6 | 6 |
| Mean(pg/mL) | 0.66 | 1.52 | 7.09 | 31.43 | 46.77 |
| Standard deviation | 0.01 | 0.03 | 0.56 | 0.66 | 1.41 |
| Coefficient of Variation (%) | 1.26% | 2.16% | 7.96% | 2.10% | 3.01% |
| Lot 2 | LLOQ | LQC | MQC | HQC | ULOQ |
| Number of Replicates | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Mean(pg/mL) | 0.83 | 1.66 | 6.49 | 33.75 | 45.82 |
| Standard deviation | 0.02 | 0.07 | 0.21 | 1.52 | 0.36 |
| Coefficient of Variation (%) | 2.07% | 4.24% | 3.25% | 4.50% | 0.78% |
| Lot 3 | LLOQ | LQC | MQC | HQC | ULOQ |
| Number of Replicates | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Mean(pg/mL) | 3.01 | 7.97 | 24.27 | 129.35 | 193.35 |

| | | | | | |
|---|---|---|---|---|---|
| Standard deviation | 0.25 | 0.49 | 2.21 | 10.63 | 4.60 |
| Coefficient of Variation (%) | 8.24% | 6.14% | 9.10% | 8.22% | 2.38% |

Inter-plate Precision

Five samples of known concentration were tested six times with each of the three batches of kits and the coefficient of variation (CV) of the concentration was calculated.

Inter-Assay: CV<15%

| | LLOQ | LQC | MQC | HQC | ULOQ |
|---|---|---|---|---|---|
| Number of Replicates | 18 | 18 | 18 | 18 | 18 |
| Mean(pg/mL) | 3.07 | 7.43 | 25.17 | 131.47 | 199.06 |
| Standard deviation | 0.08 | 0.11 | 0.45 | 1.66 | 0.85 |
| Coefficient of Variation (%) | 11.32% | 7.35% | 6.77% | 5.00% | 1.83% |

## Recovery

Matrices listed below were spiked with certain level of IL-2 and the recovery rates were calculated by comparing the measured value to the expected amount of IL-2 in samples.

| Sample Type | Recovery Range (LQC, %) | Recovery Range (MQC, %) | Recovery Range (HQC, %) |
|---|---|---|---|
| Serum(n=5) | 87.73-101.03 | 88.13-104.07 | 96.29-104.29 |
| Plasma(n=5) | 82.09-76.95 | 95.98-104.28 | 99.62-102.79 |
| Cell lysates(n=5) | 108.52-116.77 | 94.63-98.78 | 101.28-11.23 |
| Cell culture supernatants(n=5) | 95.1-110.03 | 97.12-108.12 | 108.47-114.12 |

## Linearity

Five samples were spiked with known concentrations of target proteins, and serially diluted 2-64 times. The read-out (pg/mL) for each measurement was multiplied by its respective dilution factor and the mean concentration of each sample was calculated from all values within the calibrator curve quantification range. Linearity was calculated for each measurement by relating it to its respective mean.

| Dilution | | Serum(n=5) | Cell supernatant(n=5) |
|---|---|---|---|
| 1：2 | Recovery | 108% | 97% |
| 1：4 | Recovery | 96% | 108% |
| 1：8 | Recovery | 91% | 92% |
| 1：16 | Recovery | 105% | 95% |
| 1：32 | Recovery | 98% | 103% |
| 1：64 | Recovery | 116% | 117% |

## CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human IL-2 produced at ABclonal.

The NIBSC/WHO Interleukin-2 International Standard 86/500 (IL-2, Human rDNA derived) was evaluated in this kit. The dose response curve of the International Standard ((86/500) parallels the kit standard curve. To convert sample values obtained with the Human IL-2 kit to approximate NIBSC 86/500 units, use the equation below.

NIBSC/WHO (86/500) approximate value (U/mL) =0.012 × Human IL-2 value (pg/mL)

## SAMPLE VALUES

**Serum/Plasma**- Samples from apparently healthy volunteers were evaluated for the presence of human IL-2 in this assay. No medical histories were available for the donors used in this study.

| Sample Type (n=10) | Mean of Detectable (pg/mL) | % Detectable | Range (pg/mL) |
|---|---|---|---|
| Serum | ND | 10% | ND-1.4 |

ND=Non-detectable

**Cell Culture Supernates** - Human T-lymphocyte leukemia cells (1 x $10^6$ cells/mL)

were cultured in RPMI supplemented with 40 nM PMA + 2 uM A23187 + 300 ng/mL BFA (delayed for 45 min.) . PBMCs were left untreated or treated with 10 ug/mL PHA for 5 days before collecting conditioned media.

Aliquots of the cell culture supernates were removed and

assayed for levels of human IL-2.

| Condition | Day 5 (pg/mL) |
| --- | --- |
| Unstimulated | 80.14 |
| Stimulated | > 2000 |

## Trouble Shooting

| Problem | Possible Cause | Solution |
|---|---|---|
| High Background | Insufficient washing | Sufficiently wash plates as required. Ensure appropriate duration and number of washes. Ensure appropriate volume of wash buffer in each well. |
| | Incorrect incubation procedure | Check whether the duration and temperature of incubation are set up as required. |
| | Cross-contamination of samples and reagents | Be careful of the operations that could cause cross-contamination. Use fresh reagents and repeat the tests. |
| No signal or weak signal | Incorrect use of reagents | Check the concentration and dilution ratio of reagents. Make sure to use reagents in proper order. |
| | Incorrect use of microplate reader | Warm the reader up before use. Make sure to set up appropriate main wavelength and correction wavelength. |
| | Insufficient colour reaction time | Optimum duration of colour reaction should be limited to 15-25 minutes. |

| | | |
|---|---|---|
| | Read too late after stopping the colour reaction | Read the plate in 5 minutes after stopping the reaction. |
| | Matrix effect of samples | Use positive control. |
| Too much signal | Contamination of TMB substrate | Check if TMB substrate solution turns blue. Use new TMB substrate solution. |
| | Plate sealers reused | Use a fresh new sealer in each step of experiments. |
| | Protein concentration in sample is too high | Do pre-test and dilute samples in optimum dilution ratio. |
| Poor Duplicates | Uneven addition of samples | Check the pipette. Periodically calibrate the pipette. |
| | Impurities and precipitates in samples | Centrifuge samples before use. |
| | Inadequate mixing of reagents | Mix all samples and reagents well before loading. |

*For research purposes only. Not for therapeutic or diagnostic purposes.