

# AviBion Human Adiponectin (Acrp30) ELISA Kit

## User Manual

**REF** : ADIP025

**RUO**  96

**Regulatory Status:** For research use only (RUO)

*Please contact Orgenium's customer service representatives for inquiries, feedback or non-conforming products.*

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## 1. INTENDED USE

Orgenium Laboratories' Adiponectin (Acrp30) ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human Adiponectin in cell culture supernatants, plasma and human serum.

## 2. INTRODUCTION

Adiponectin, a collagen-like plasma protein produced specifically by adipose tissue, is abundantly present in the circulation. Adiponectin was first identified in the mid-1990s and named as AdipoQ, apM1 (adipose most abundant gene transcript 1), GBP28 (gelatin-binding protein), or Acrp30 (adipocyte complement-related protein 30) (1 - 4). Human adiponectin is encoded by the ADIPOQ gene (previously named APM1 or ACDC), which spans 17 kb on chromosome locus 3q27 (5, 6). Interestingly, human chromosome 3q27 has been identified as a region carrying susceptibility genes for type 2 diabetes and metabolic syndrome (7, 8). The gene for human adiponectin contains three exons, with the start codon in exon 2 and stop codon in exon 3 (5, 6).

Plasma concentrations of adiponectin are reduced in the setting of obesity (9), in patients with non-insulin dependent diabetes mellitus (10), acute myocardial infarction (11, 12), essential hypertension (13), inflammation and atherosclerosis (14). In addition, increased adiponectin levels lead to nephrotic syndrome (15). Adiponectin has an important role in glucose metabolism and is negatively correlated with body fat amount in adults (16, 17).

Orgenium Laboratories' human Adiponectin ELISA test is a solid-phase ELISA assay designed to measure the quantitative amount of total (low, middle, and high molecular weight) human adiponectin in cell culture supernates, serum and plasma. This assay employs an antibody specific for human adiponectin coated on a 96-well plate. Standards, samples and biotinylated anti-human adiponectin are pipetted into the wells and adiponectin present in a sample is captured by the antibody immobilized to the wells and by the biotinylated adiponectin-specific detection antibody. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed. Following this second wash step, TMB substrate solution is added to the wells, resulting in color development proportional to the amount of Adiponectin bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.



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### 3. CONTENTS OF THE KIT

Test components	Amount/Volume
<p><b>96 Well Plate with 12 Strips</b> Break-apart microtiter test strips each with 8 Adiponectin antibody coated single wells Ready for use</p>	1 frame
<p><b>Adiponectin Standard 30 ng/ml</b> Lyophilized &amp; Stabilized Recombinant Human Adiponectin (see label for stock concentration) Add 1 ml of "Sample Diluent" before use.</p>	2x1 ml
<p><b>Biotinylated Adiponectin antibody.</b> Ready for use.</p>	10 ml
<p><b>HRP-Conjugated Avidin.</b> Ready for use.</p>	12 ml
<p><b>20x Wash Buffer concentrate</b> (sufficient for 1000ml) <b>Dilute 1:20</b></p>	50 ml
<p><b>Sample Diluent</b> Ready for use</p>	2x 100 ml
<p><b>Stop solution</b> 2 N H<sub>2</sub>SO<sub>4</sub> Ready for use</p>	8 ml
<p><b>TMB-Substrate</b> Ready for use</p>	8 ml

#### 4. STORAGE AND STABILITY

Reagent	Storage	Stability
<b>Antibody coated 96 well plates with 12 strips.</b>  <b>Break-apart microtiter test strips each with 8 antibody coated single wells</b>	Store at 2-8°C in closed aluminum pouch with desiccant  <b>Strips which are not used must be stored in the re-sealable aluminum pouch in humidity free and airtight conditions</b>	3 months after opening
<b>Adiponectin Standard</b> Lyophilized	Store at 2-8°C <i>Avoid contamination (Use clean sterile tips)</i>	Until date of kit expiry in lyophilized format. At least 3 weeks after dissolving with sample diluent.
<b>Biotinylated antibody.</b> Ready for use.	Store at 2-8°C <i>Avoid contamination (Use clean sterile tips)</i>	3 months after opening
<b>HRP-Conjugated Avidin.</b> Ready for use.	Store at 2-8°C <i>Avoid contamination (Use clean sterile tips)</i>	3 months after opening
<b>Sample Diluent</b>	Store at 2-8°C <i>Avoid contamination (use clean sterile tips or pipettes)</i>	3 months after opening
<b>20x Concentrated Wash Buffer</b>  <b>Diluted Wash Buffer</b>	Store at Room Temperature.  1x working dilution  <i>Bottles used for the working dilution should be cleaned regularly, discard cloudy solutions</i>	Until expiry date at room temperature  3 working days at room temperature or 2 weeks at +4°C.
<b>TMB-Substrate Solution</b>	Ready for use solution at 2-8°C, protected from light!  <i>Avoid contamination (Use clean sterile tips)</i>	Until expiry date (written on the bottle).
<b>Stop Solution</b>	Store at 2-8°C. May also be stored at Room Temperature	Until expiry date

## 5. ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes (2 µl to 1 ml volumes).
- Multi-channel pipette (25 µl to 350 µl) 12 and 8 channel pipets.

### Recommended for manual washings and reagent dispensing.

- Adjustable 1-25 ml pipettes for reagent preparation.
- 100 ml and 1 liter graduated cylinders.
- Microplate washer or 12 well Multichannel pipet for washings.
- Absorbent paper.
- Distilled or de-ionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.
- Timer

## 6. AMOUNTS OF THE REAGENTS NEEDED TO PERFORM THE TEST

No of strips used (with 8 well each)	Reagents				
	Biotinylated antibody 50 µl/well	Avidin-HRP 50 µl/well	TMB Substrate 50 µl/well	Stop Solution 25 µl/well	Wash Buffer 300 µl/well
1 (8 wells)	500µl	500 µl	500 µL	300 µl	30 ml
2 (16 wells)	1 ml	1ml	1 ml	600 µL	55 ml
4 (32 wells)	2 ml	2 ml	2 ml	1.2 ml	110 ml
6 (48 wells)	3 ml	3 ml	3 ml	1.8 ml	165 ml
8 (64 wells)	4 ml	4 ml	4 ml	2.4 ml	220 ml
12 (96 wells)	6 ml	6 ml	6 ml	4 ml	350 ml



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## 7. REAGENT AND SAMPLE PREPARATION

1. Bring all reagents and samples to room temperature (18-25°C) before use.
2. **Antibody coated plate:** Before opening the foil pouch, determine the number of strips required to test the desired number of samples plus 16 wells needed for running standards and blanks in duplicate. Remove non-used strips from the plate-frame and return them to the foil pouch containing the desiccant for up to 1 month at 2-8°C.

### 3. Dilution of test standard:

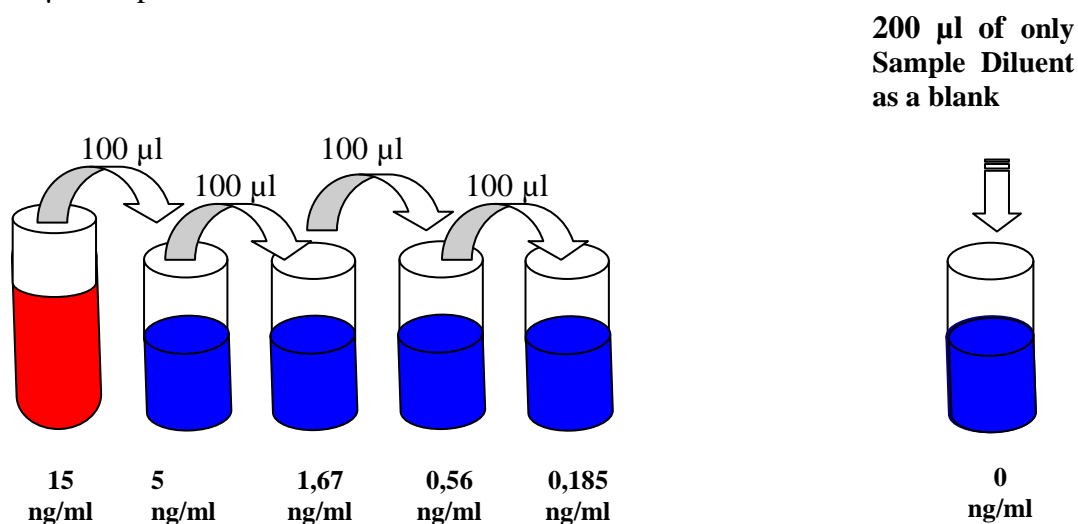
Dissolve the lyophilised Adiponectin standard with 1 ml of "Sample Diluent". Adiponectin standard is stable for at least 3 weeks after dissolving. To obtain a standard curve dilute as follows:

- a) Add 125 µl of Adiponectin standard and 125 µl of sample diluent the first tube to obtain 15 ng/ml Adiponectin standard.
- b) Add 200 µl of dilution buffer to other 4 wells and start 3 fold serial dilutions in dilution tubes as described in the figure.
- c) Add only sample diluent to tube no 6 using a clean tip.

125 µl of Adiponectin standard

+

125 µl Sample Diluent



4. **Sample preparation and dilution:** Sample diluent is used for dilution of all samples (serum/plasma samples, culture supernatants and urine) requiring dilution. Store and dilute all samples in tubes or plates made of material with low binding surface, such as polypropylene. Prior to the assay, frozen samples



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should be thawed as quickly as possible in tap water (18-25°C). Do not use 37°C or 56°C water bath for this purpose.

5.

• **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. **Dilute plasma samples 1:1000 with Sample Diluent. We recommend to perform the dilution in two steps, e.g. first diluting 1:100 (5µl sample+495µl Sample Diluent, and further 1:10 (100µl diluted sample +900µl Sample Diluent).** Do not use grossly haemolyzed or lipemic specimens. Undiluted samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

• **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. **Dilute serum samples 1:1000 with Sample Diluent. We recommend to perform the dilution in two steps, e.g. first diluting 1:100 (5µl sample+495µl Sample Diluent, and further 1:10 (100µl diluted sample +900µl Sample Diluent).** Undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

• **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. **Dilute Cell Culture Supernatants 1:10 with Sample Diluent (e.g 10 µL Sample + 90 µL diluent).** Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

**Samples with high absorbance values:** Samples that exceed the measuring range should be diluted 1:2 or 1:5 with Sample Diluent, and measured again. Samples with absorbance values >1.900 can be serially diluted 1:1000, 1:2000 and 1:4000. The dilution factor must be taken in account when calculating the results.

6. **Wash Buffer:** If the 20x concentrated Wash Buffer contains visible crystals, warm it at 37°C and mix gently until dissolved. Dilute 25 ml of Wash Buffer Concentrate with de-ionized or distilled water to yield 500 ml of 1x Wash Buffer.

7. Vortex mix **Biotinylated antibody** solution gently before use.



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8. Vortex mix **peroxidase (HRP) labeled avidin** gently before use.

**Caution:** TMB substrate (Tetramethylbenzidine) and the Stop solution ( $H_2SO_4$ ) are toxic or corrosive and should be handled with care. Use gloves during handling.



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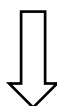
## 8. TEST PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards.

Dilute samples as follows;

**Serum & Plasma:** 1:1000 diluted samples

**Cell Culture Supernatants:** 1:10 diluted samples



2. Add 50 µl of each diluted sample, diluted standards (starting from 15ng/mL) and sample diluent as a blank into the appropriate wells of the strips.

Incubate 1 hour at room temperature.



Wash 5 x with 1x wash buffer

3. Add 50 µl ready for use biotin antibody promptly to each well.

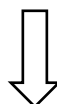
Incubate 1 hour at room temperature.



Wash 5 x with 1x wash buffer

4. Add 50 µl ready for use HRP-Streptavidin solution.

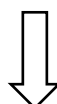
Incubate 30 minutes at room temperature.



Wash 5 x with 1x wash buffer

5. Add 50 µl TMB One-Step Substrate Reagent to each well.

Incubate 20 minutes at room temperature.



6. Add 25 µl Stop Solution to each well.

Read at 450 nm against \*630 nm immediately.

*\*Correcting for optical imperfections in the microplates by subtracting  $A_{630\text{ nm}}$  is recommended, but not an essential procedure.*



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## 9. PROCEDURAL NOTES/LAB QUALITY CONTROL

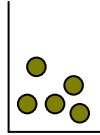
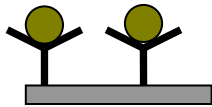
- When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
- Microtiter plates should be allowed to come to room temperature before opening the aluminum pouches.
- Once the desired number of strips has been removed, immediately reseal the pouch and store at 2 - 8°C to maintain plate integrity. Protect from humidity.
- Samples should be collected in pyrogen/endotoxin-free tubes.
- Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
- When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
- It is recommended that all standards, controls and samples be run in duplicate.
- Samples that contain >15ng/mL Adiponectin should be diluted serially with Sample Diluent.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
- Cover or cap all reagents when not in use.
- Do not use reagents after the kit expiration date.
- Read absorbances within 15 minutes of assay completion.
- In-house controls should be run with every assay.
- All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. **Never** insert absorbent paper directly into the wells.
- Because *TMB Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

## TEST PRINCIPLE



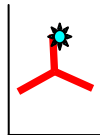
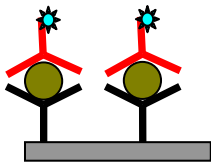
Adiponectin Antibody coated test well

### STEP 1



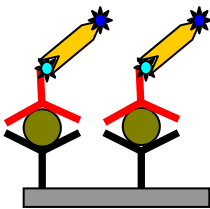
Add 50µL of diluted Adiponectin standards and samples to test wells

### STEP 2



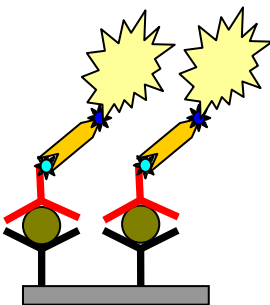
Add 50 µL of Biotinylated Adiponectin antibody to test well

### STEP 3

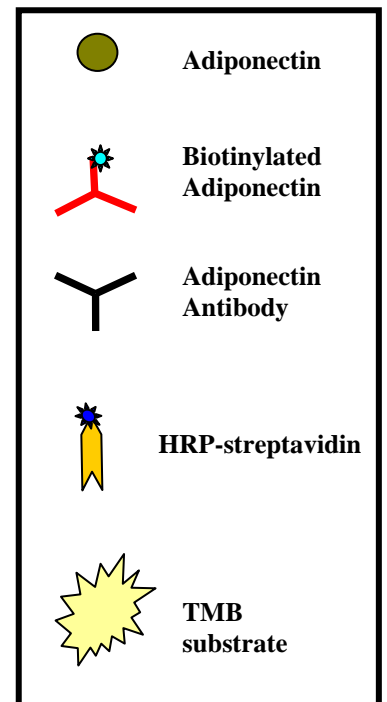


Add 50 µL of HRP-Streptavidin to test

### STEP 2



Add 50 µL of TMB substrate to test well



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## 10. ASSAY PROCEDURE

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate. Leave some wells as a reagent blank (2 to 4 wells).

### FIRST STEP: STANDARD, SAMPLES AND BLANK + BIOTINYLATED-ANTIBODY

2. Pipette 50 µl of sample and 50 µl of each diluted standard starting from 15ng/mL into appropriate wells (see page 7). Pipette 50 µl of sample diluent to the wells which will be used as a blank. Incubate for one hour, wash 5 times with 1x Wash Solution (300 µl each).

3. Add 50 µl of Green colored Biotinylated detection antibody to all wells containing standards and samples. Incubate for one hour, wash 5 times with 1x Wash Solution (300 µl each).

To wash: Empty plate contents. Use a multi-channel pipette to fill each well with 300 µl of wash buffer, then empty plate contents again. Repeat procedure 4 additional times for a total of FIVE washes. Gently blot plate onto paper towels or other absorbent material.

**Note:** For automated washing, aspirate all wells and wash 5 times with Wash Buffer. Blot plate onto paper towels or other absorbent material. Never let reaction wells dry. Continue to the next step without delay or interruption.

### SECOND STEP: STREPTAVIDIN-HRP

4. Add 50 µl of prepared Streptavidin-HRP solution (Ready to use) to each well. Incubate for 30 minutes at room temperature.

5. Wash 5 times with 1x wash solution (300 µl each).

### THIRD STEP: TMB SUBSTRATE

6. Add 50 µl of TMB ready to use substrate reagent to each well.

Incubate for 20 minutes at room temperature in the dark.

### FOURTH STEP: STOP REACTION AND READ THE PLATE

7. Add 25 µl of Stop Solution to each well. Read at 450 nm within 15 minutes.

*Correcting for optical imperfections in the microplates by subtracting  $A_{630\text{ nm}}$  is recommended, but not an essential procedure.*



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## FIFTH STEP: READING AND CALCULATION

8. Calculate the mean of reagent blank absorbance values and subtract it from all test well values (standard and test samples). Mean reagent blank absorbance value at 450 nm should be less than 0.200.
9. Calculate your results against standard.

### 11. CALCULATION OF RESULTS

The standard curve must be determined individually for each experiment. Correct each absorbance value of all standards by subtracting from it the O.D. value of the reagent blank (BI = only sample diluent). Calculate the mean absorbance value for each standard from the duplicates.

The standard curve is used to determine the amount of adiponectin in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding adiponectin concentration (ng/mL) on the horizontal (X) axis.

Construct the standard curve using graph paper or statistical software.

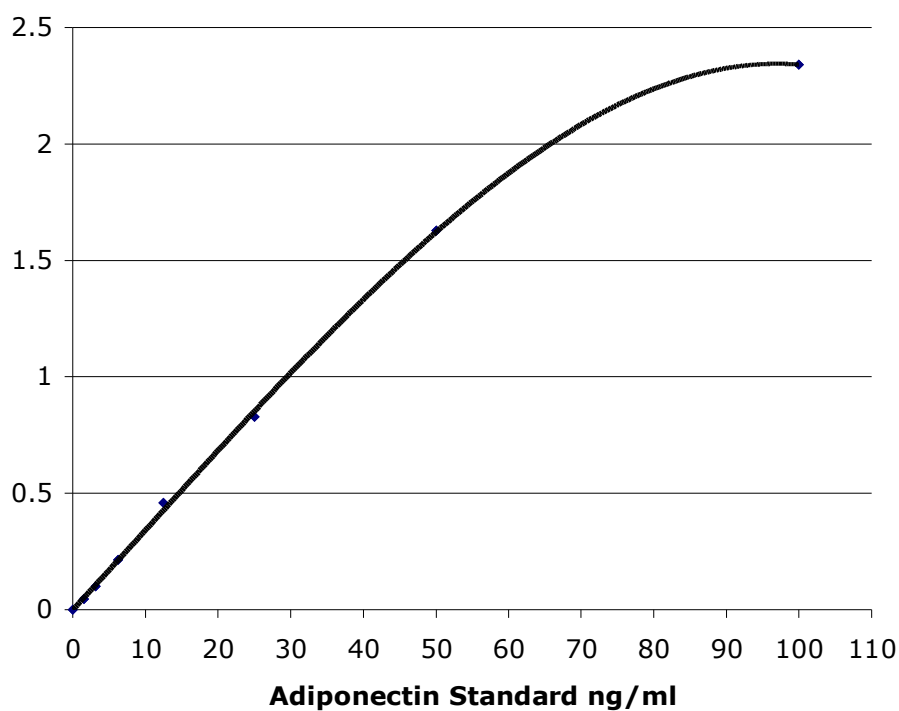
If samples generate values higher than the highest standard, dilute the samples with sample diluent and repeat the assay. Note that the concentration read from the standard curve must be multiplied by the dilution factor.

**Multiply the plasma or serum value by the dilution factor of 1000 and the cell-culture value by the dilution factor of 10.**

## 12. TYPICAL DATA

The following graph was obtained for the various Adiponectin standards over the range of 0 to 15 ng/mL.

*Please note: This graph is an example only. A standard curve should be generated each time the assay is performed. Do not use these values in your calculations.*



### 13. TEST PERFORMANCE

	<b>Adiponectin</b>
<b>Assay range</b>	0.185 -15ng/ml
<b>Standard curve points</b>	15, 5, 1.67, 0.56, 0.185 and 0 ng/ml.
<b>Intra-Assay-Precision</b>	≤10%
<b>Inter-Assay-Precision</b>	≤12%
<b>Inter-Lot-Precision</b>	≤12%
<b>Cross-Reactivity</b>	No cross-reactivity was observed with the following recombinant human proteins: IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, TNF α, TARC
<b>Specificity</b>	Recognizes human Adiponectin.
<b>Sensitivity</b>	<3 ng/ml.

### 14. REFERENCES

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## LIABILITY

This kit is intended for research use only by personnel trained and qualified to carry out diagnostic or research activities.

If the recipient of this kit passes it on in any way to a third party, this instruction must be enclosed, and said recipient shall at own risk secure in favor of Orgenium Laboratories all limitations of liability herein.

Orgenium Laboratories shall not be responsible for any damages or losses due to using the kit in any way other than as expressly stated in these Instructions.

The liability of Orgenium Laboratories shall in no event exceed the commercial value of the kit.

Orgenium Laboratories shall under no circumstances be liable for indirect, special or consequential damages, including but not limited to loss of profit.



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## 15. TROUBLESHOOTING

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
<b>Poor standard Curve</b>	<ol style="list-style-type: none"> <li>1. Inaccurate pipetting or pipetting error</li> <li>2. Improper standard dilution</li> </ol>	<p>Check pipettes and calibrate regularly.</p> <p>Vortex the stock before use and dilute carefully in an eppendorf tube.</p>
<b>Low signal</b>	<ol style="list-style-type: none"> <li>1. Shorter incubation than recommended</li> <li>2. Inadequate reagent volumes or improper dilution or pipetting error</li> </ol>	<p>Ensure sufficient incubation time;</p> <p>Check pipettes and ensure correct performance.</p>
<b>Large CV</b>	Inaccurate pipetting and drying of wells during test procedure.	<p>Check pipettes</p> <p>Fill the wells promptly with wash buffer and reagents.</p>
<b>High background</b>	<ol style="list-style-type: none"> <li>1. Plate is insufficiently washed</li> <li>2. Contaminated wash Buffer</li> <li>3. Wash buffer volume is less than advised</li> </ol>	<p>Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed and clean.</p> <p>Make a fresh wash buffer</p> <p>Use 300µl per well</p>
<b>Low sensitivity</b>	<ol style="list-style-type: none"> <li>1. Improper storage of the ELISA kit</li> <li>2. Stop solution</li> <li>3. Contamination of reagents</li> </ol>	<p>Store test kit components as advised in this user manual. Keep substrate solution protected from light.</p> <p>Stop solution should be added to each well before measure.</p> <p>Use clean sterile tips. Discard contaminated reagents.</p>