

AdipoGenTM 

MANUAL

Adiponectin (human) ELISA Kit

For research use only. Not for diagnostic use.

Version 2 (14-March-2011)

Cat. No. AG-45A-0001EK-KI01

www.adipogen.com

Table of Contents

| | |
|--|------------------|
| 1. Intended Use | 3 |
| 2. Introduction | 3 |
| 3. General References | 3 |
| 4. Assay Principle | 4 |
| 5. Handling & Storage | 4 |
| 6. Kit Components | 4 |
| 7. Materials Required but <i>Not</i> Supplied | 5 |
| 8. General ELISA Protocol | 6 |
| 8.1. Preparation and Storage of Reagents | 6 |
| 8.2. Sample Collection, Storage and Dilution | 7 |
| 8.3. Assay Procedure (Checklist) | 8 |
| 9. Calculation of Results | 9 |
| 10. Typical Data | 9 |
| 11. Performance Characteristics | 10-12 |
| 12. Technical Hints and Limitations | 13 |
| 13. Troubleshooting | 14 |
| 14. Assay Flow Chart | 15 |
| <hr/> | |
| Product Specific References | Backcover |

1. Intended Use

The Adiponectin (human) ELISA Kit is to be used for the *in vitro* quantitative determination of human Adiponectin in serum, plasma, urine and cell culture supernatant. This ELISA Kit is for research use only.

2. Introduction

Adipocytes express a variety of adipocytokines that function in the homeostatic control of glucose and lipid metabolism. Insulin regulates secretion of many of these adipocytokines in response to changes in energy balance. Adiponectin is a 244-amino acid protein with high structural homology to collagen VIII, collagen V, complement C1q (Ref. 1 and 2), and TNF (Ref.3), which is exclusively and abundantly expressed in white adipose tissue. Plasma adiponectin concentrations have found to be decreased in obesity and/or type-2 diabetes, resulting in the conditions commonly associated with insulin resistance and hyper-insulinemia (Ref. 4-5). Therefore, measurement of the plasma level of adiponectin may be important for understanding diagnosis or prognosis of onset of these diseases.

3. General References

- (1) A novel serum protein similar to C1q, produced exclusively in adipocytes: P.E. Scherer, et al.; J. Biol. Chem. 270, 26746 (1995)
- (2) AdipoQ is a novel adipose-specific gene dysregulated in obesity: E. Hu, et al.; J. Biol. Chem. 18, 10697 (1996)
- (3) The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis: L. Shapiro, et al.; Curr. Biol. 12, 335 (1998)
- (4) Plasma resistin concentrations are elevated in Individuals with Type-2 diabetes mellitus: B.S. Youn, et al.; J. Cli. Endo. Meta. 89, 150 (2004)
- (5) Genetic association study of adiponectin polymorphisms with risk of type 2 diabetes mellitus in Korean population: Y. Y. Lee, et al.; Diabet. Med. 22, 569 (2004)

4. Assay Principle

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of human adiponectin in biological fluids. A monoclonal antibody specific for adiponectin has been precoated onto the 96-well microtiter plate. Standards and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, adiponectin is recognized by the addition of a purified polyclonal antibody specific for adiponectin (Detection Antibody). After removal of excess polyclonal antibody, HRP conjugated anti-rabbit IgG (Detector) is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of adiponectin in the samples.

5. Handling & Storage

- Reagent must be stored at 2-8°C when not in use.
- Plate and reagents should be at room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

6. Kit Components

| | |
|---|----------------------|
| 1 plate coated with human adiponectin Antibody | (12 x 8-well strips) |
| 1 bottle Wash Buffer 10X | (50 ml) |
| 1 bottle Diluent 5X | (50 ml) |
| 1 bottle Detection Antibody | (12 ml) |
| 1 vial Detector 100X (HRP Conjugated anti-rabbit IgG) | (150 µl) |
| 1 vial human adiponectin Standard (lyophilized) | (64 ng) |
| 1 vial human adiponectin QC sample (lyophilized) | |
| 1 bottle Substrate Solution I (TMB) | (6 ml) |
| 1 bottle Substrate Solution II (Peroxidase) | (6 ml) |
| 1 bottle Stop Solution | (12 ml) |
| 3 plate sealers (plastic film) | |

7. Materials Required but Not Supplied

- Microtiterplate reader at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Calibrated precision single and multi-channel pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

8. General ELISA Protocol

8.1. Preparation and Storage of Reagents

NOTE: Prepare just the appropriate amount of the buffers necessary for the assay.

- **Wash Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 50 ml Wash Buffer 10X + 450 ml water) to obtain Wash Buffer 1X.
- **Diluent 5X** has to be diluted with deionized water 1:5 before use (e.g. 50 ml Diluent 5X + 200 ml water) to obtain Diluent 1X.
- **Detector 100X (HRP Conjugated anti-rabbit IgG)** has to be diluted to the working concentration by adding 120 µl in 12 ml of Diluent 1X (1:100).

NOTE: The diluted Detector is used within one hour of preparation.

- **Substrate Solution I and II** have to be mixed together in equal volumes within 15 minutes of use.

NOTE: Freshly prepare just before use the Substrate Solution and protect from light!

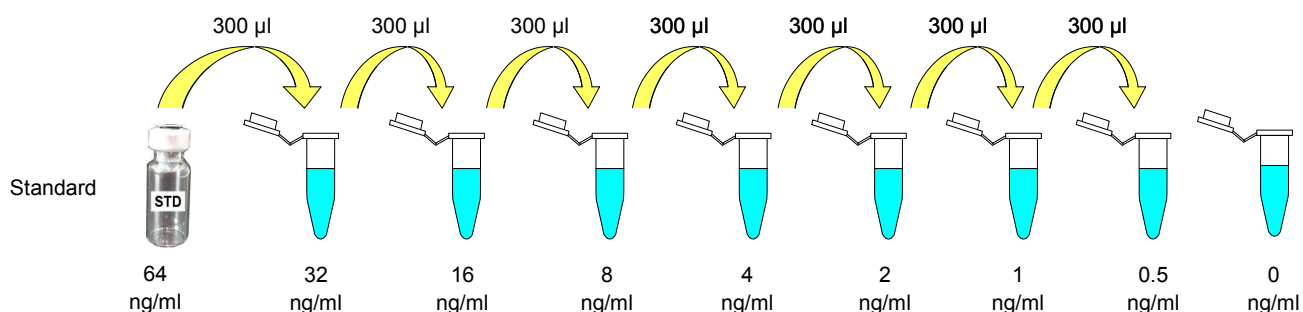
- **Human adiponectin Standard (STD)** has to be reconstituted with 1 ml of deionized water.
 - This reconstitution produces a stock solution of 64 ng/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

NOTE: The reconstituted standard is aliquoted and stored at -20°C

- Dilute the standard protein concentrate (STD) (**64 ng/ml**) in Diluent 1X. A seven-point standard curve using 2-fold serial dilutions in Diluent 1X is recommended.
- Suggested standard points are:
32 , 16 , 8 , 4 , 2 , 1 , 0.5 and 0 ng/ml.
- **Human adiponectin QC sample** has to be reconstituted with 1 ml of deionized water.
 - Refer to the Certificate of Analysis for current QC sample concentration. Mix the QC sample to ensure complete reconstitution and allow the QC sample to sit for a minimum of 15 minutes. The reconstituted QC sample is ready to use, do not dilute it.

Dilute further for the standard curve:

| To obtain | Add | Into |
|------------------|----------------------------------|----------------------|
| 32 ng/ml | 300 µl of adiponectin (64 ng/ml) | 300 µl of Diluent 1X |
| 16 ng/ml | 300 µl of adiponectin (32 ng/ml) | 300 µl of Diluent 1X |
| 8 ng/ml | 300 µl of adiponectin (16 ng/ml) | 300 µl of Diluent 1X |
| 4 ng/ml | 300 µl of adiponectin (8 ng/ml) | 300 µl of Diluent 1X |
| 2 ng/ml | 300 µl of adiponectin (4 ng/ml) | 300 µl of Diluent 1X |
| 1 ng/ml | 300 µl of adiponectin (2 ng/ml) | 300 µl of Diluent 1X |
| 0.5 ng/ml | 300 µl of adiponectin (1 ng/ml) | 300 µl of Diluent 1X |
| 0 ng/ml | 300 µl of Diluent 1X | Empty tube |



8.2. Sample Collection, Storage and Dilution

Serum : Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at $\leq -20^{\circ}\text{C}$ for later use. Avoid repeated freeze/thaw cycles.

Plasma : Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at $\leq -20^{\circ}\text{C}$ for later use. Avoid repeated freeze/ thaw cycles.

Urine : Aseptically collect the urine of the day, voided directly into a sterile container. Assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze/thaw cycles.

Serum, Plasma, Urine or Cell Culture Supernatant have to be diluted in Diluent 1X. Samples containing visible precipitates must be clarified before use.

NOTE: As a starting point, 1/2,000 dilution of serum or plasma and 1/10 dilution of urine are recommended!

8.3. Assay Procedure (Checklist)

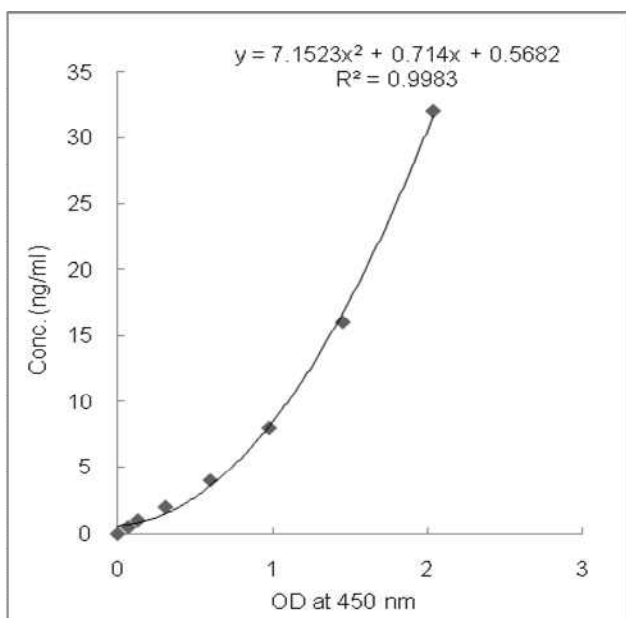
| | |
|--------------------------|--|
| <input type="checkbox"/> | <p>1. Determine the number of 8-well strips needed for the assay and insert them in the frame for current use. The extra strips should be resealed in the foil pouch bag and stored at 4°C.</p> <p>NOTE: Remaining 8-well strips coated with adiponectin antibody when opened can be stored at 4°C for up to 1 month.</p> |
| <input type="checkbox"/> | <p>2. Add 100 µl of the different standards into the appropriate wells in duplicate! At the same time, add 100 µl of diluted serum, plasma, urine or cell culture supernatant samples in duplicate to the wells (see 8.1. Preparation and Storage of Reagents and 8.2. Preparation of Samples).</p> |
| <input type="checkbox"/> | <p>3. Cover the plate with plate sealer and incubate for 1 hour at 37°C.</p> |
| <input type="checkbox"/> | <p>4. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.</p> |
| <input type="checkbox"/> | <p>5. Add 100 µl to each well of the Detection Antibody.</p> |
| <input type="checkbox"/> | <p>6. Cover the plate with plate sealer and incubate for 1 hour at 37°C.</p> |
| <input type="checkbox"/> | <p>7. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.</p> |
| <input type="checkbox"/> | <p>8. Add 100 µl to each well of the diluted Detector (see 8.1. Preparation and Storage of Reagents).</p> |
| <input type="checkbox"/> | <p>9. Cover the plate with plate sealer and incubate for 1 hour at 37°C.</p> |
| <input type="checkbox"/> | <p>10. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.</p> |
| <input type="checkbox"/> | <p>11. Add 100 µl to each well of mixed substrate solution.</p> |
| <input type="checkbox"/> | <p>12. Allow the color reaction to develop at room temperature (RT°C) in the dark for 20 minutes.</p> |
| <input type="checkbox"/> | <p>13. Stop the reaction by adding 100 µl of Stop Solution. Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.</p> |
| | ! CAUTION: CORROSIVE SOLUTION! |
| <input type="checkbox"/> | <p>14. Measure the OD at 450 nm in an ELISA reader within 30 minutes.</p> |

9. Calculation of Results

- Average the duplicate readings for each standard, QC and sample and subtract the average blank value (obtained with the 0 ng/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding adiponectin concentration (ng/ml) on the vertical (Y) axis (see 10. TYPICAL DATA).
- Calculate the adiponectin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
- If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of human adiponectin in the samples.

10. Typical Data

The following data are obtained using the different concentrations of standard as described in this protocol:



| Standard hAdiponectin (ng/ml) | Optical Density (mean) |
|-------------------------------|------------------------|
| 32 | 2.036 |
| 16 | 1.453 |
| 8 | 0.978 |
| 4 | 0.598 |
| 2 | 0.309 |
| 1 | 0.131 |
| 0.5 | 0.068 |
| 0 | 0 |

Figure: Standard curve

11. Performance Characteristics

A. Sensitivity (Limit of detection):

The lowest level of adiponectin that can be detected by this assay is 100 pg/ml. **NOTE:** *The Limit of detection was measured by adding two standard deviations to the mean value of 50 zero standard.*

B. Assay range: 0.5 ng/ml – 32 ng/ml

C. Specificity:

This ELISA is specific for the measurement of natural and recombinant human adiponectin. It does not cross-react with mouse adiponectin, rat adiponectin, human resistin, human RELM- β , human leptin, human TNF- α , human IL-6.

D. Intra-assay precision:

Four serum samples of known concentrations of human adiponectin were assayed in replicates 5 times to test precision within an assay.

| Samples | Means (μ g/ml) | SD | CV (%) | n |
|---------|---------------------|------|--------|---|
| 1 | 1.86 | 0.07 | 3.82 | 5 |
| 2 | 5.90 | 0.23 | 3.84 | 5 |
| 3 | 8.50 | 0.28 | 3.31 | 5 |
| 4 | 23.36 | 0.69 | 2.97 | 5 |

Five urine samples of known concentrations of human adiponectin were assayed in replicates 6 times to test precision within an assay.

| Samples | Means (ng/ml) | SD | CV (%) | n |
|---------|---------------|------|--------|---|
| 1 | 30.40 | 2.53 | 8.31 | 6 |
| 2 | 5.42 | 0.41 | 7.54 | 6 |
| 3 | 10.28 | 0.34 | 3.33 | 6 |
| 4 | 95.83 | 3.38 | 3.53 | 6 |
| 5 | 69.94 | 1.48 | 2.12 | 6 |

E. Inter-assay precision:

Four serum samples of known concentrations of human adiponectin were assayed in 5 separate assays to test precision between assays.

| Samples | Means (µg/ml) | SD | CV (%) | n |
|---------|---------------|------|--------|---|
| 1 | 2.50 | 0.13 | 5.15 | 5 |
| 2 | 7.78 | 0.43 | 5.50 | 5 |
| 3 | 11.10 | 0.44 | 3.97 | 5 |
| 4 | 24.82 | 0.70 | 2.84 | 5 |

Five urine samples of known concentrations of human adiponectin were assayed in 3 separate assays to test precision between assays.

| Samples | Means (ng/ml) | SD | CV (%) | n |
|---------|---------------|------|--------|---|
| 1 | 28.16 | 1.81 | 6.44 | 3 |
| 2 | 5.94 | 0.23 | 3.93 | 3 |
| 3 | 3.91 | 0.36 | 9.09 | 3 |
| 4 | 108.91 | 8.26 | 7.58 | 3 |
| 5 | 76.13 | 7.38 | 9.69 | 3 |

F. Recovery:

The recovery of adiponectin spiked to four different levels in five different serum samples and four different urine samples throughout the range of assay was evaluated.

| Samples | Average recovery (%) | Range (%) | |
|--------------|----------------------|-----------|--------|
| Serum | 1 | 99.6 | 96-105 |
| | 2 | 99.8 | 96-104 |
| | 3 | 100.2 | 97-102 |
| | 4 | 92.5 | 88-95 |
| | 5 | 91.8 | 86-100 |
| Urine | 1 | 101.9 | 96-105 |
| | 2 | 97.9 | 96-104 |
| | 3 | 91.2 | 88-95 |
| | 4 | 84.7 | 80-90 |

G. Linearity:

Different human serum samples containing adiponectin were diluted several fold (1/1,000 to 1/4,000) and the measured recoveries ranged from 87% to 102%.

| Samples | Sample Dilution | Expected (µg/ml) | Observed (µg/ml) | % of Expected |
|---------|-----------------|------------------|------------------|---------------|
| 1 | 1 : 1,000 | 13.61 | 13.61 | 100 |
| | 1 : 2,000 | 6.81 | 6.64 | 97.6 |
| | 1 : 4,000 | 3.40 | 2.97 | 87.2 |
| 2 | 1 : 1,000 | 15.89 | 15.89 | 100 |
| | 1 : 2,000 | 7.94 | 8.09 | 101.9 |
| | 1 : 4,000 | 3.97 | 3.76 | 94.7 |
| 3 | 1 : 1,000 | 11.51 | 11.51 | 100 |
| | 1 : 2,000 | 5.76 | 5.77 | 100.2 |
| | 1 : 4,000 | 2.88 | 2.51 | 87.1 |

Different human urine samples containing adiponectin were diluted several fold (1/5 to 1/10) and the measured recoveries ranged from 97% to 105%.

| Samples | Sample Dilution | Expected (ng/ml) | Observed (ng/ml) | % of Expected |
|---------|-----------------|------------------|------------------|---------------|
| 1 | 1 : 5 | 3.85 | 3.85 | 100 |
| | 1 : 10 | 1.92 | 2.00 | 104.2 |
| 2 | 1 : 5 | 5.14 | 5.14 | 100 |
| | 1 : 10 | 2.57 | 2.66 | 103.8 |
| 3 | 1 : 5 | 45.79 | 45.79 | 100 |
| | 1 : 10 | 22.89 | 23.56 | 102.9 |

H. Expected values:

Adiponectin levels range in plasma and serum from **4 to > 15 µg/ml** (from healthy donors).

Adiponectin levels range in urine from **3 to > 15 ng/ml** (from healthy donors).

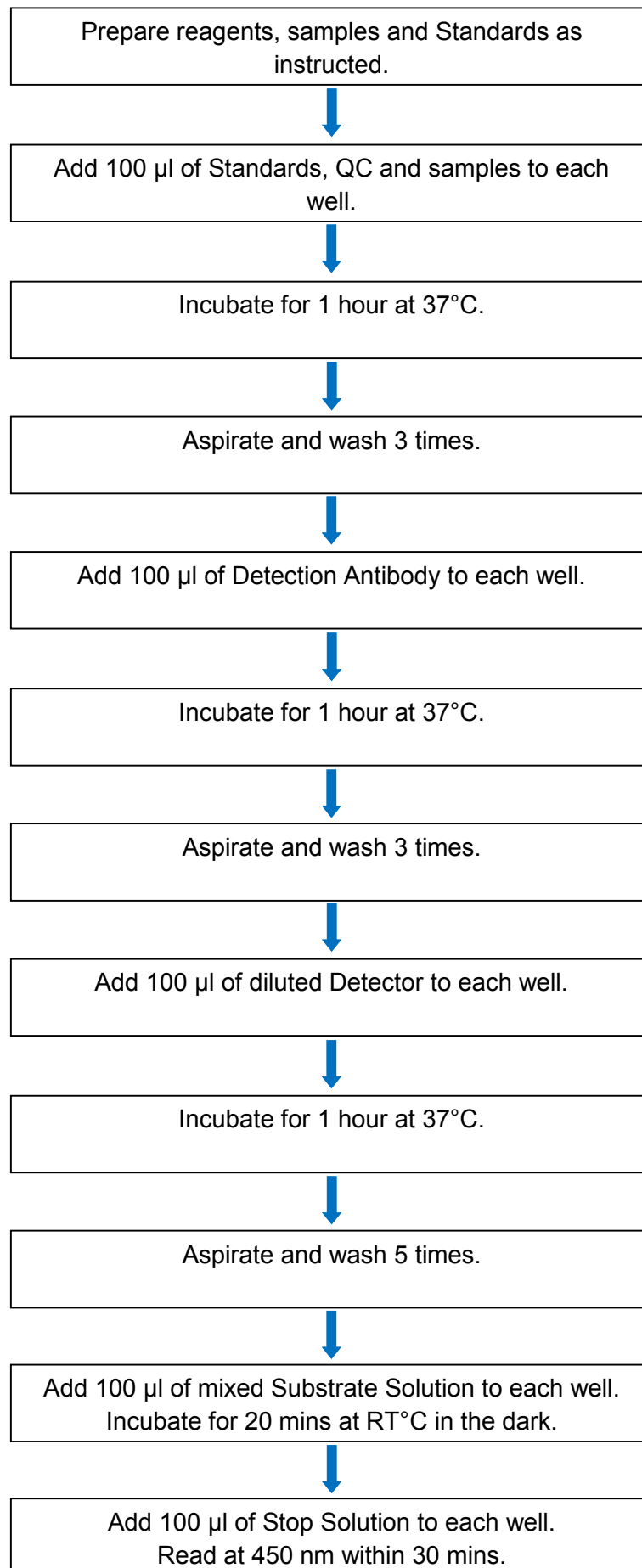
12. Technical Hints and Limitations

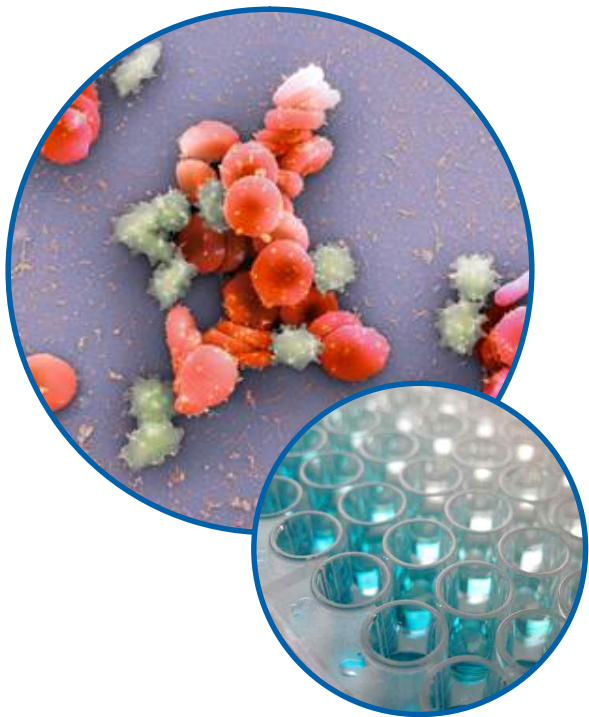
- It is recommended that all standards, QC sample and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 8-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep Substrate Solution protected from light.
- The Stop Solution consists of phosphoric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

13. Troubleshooting

| PROBLEM | POSSIBLE CAUSES | SOLUTIONS |
|--------------------------|------------------------------------|--|
| No signal or weak signal | Omission of key reagent | Check that all reagents have been added in the correct order. |
| | Washes too stringent | Use an automated plate washer if possible. |
| | Incubation times inadequate | Incubation times should be followed as indicated in the manual. |
| | Plate reader settings not optimal | Verify the wavelength and filter setting in the plate reader. |
| | Incorrect assay temperature | Use recommended incubation temperature. Bring substrates to room temperature before use. |
| High background | Concentration of detector too high | Use recommended dilution factor. |
| | Inadequate washing | Ensure all wells are filling wash buffer and are aspirated completely. |
| Poor standard curve | Wells not completely aspirated | Completely aspirate wells between steps. |
| | Reagents poorly mixed | Be sure that reagents are thoroughly mixed. |
| Unexpected results | Omission of reagents | Be sure that reagents were prepared correctly and added in the correct order. |
| | Dilution error | Check pipetting technique and double-check calculations. |

14. Assay Flow Chart





Product Specific References:

1. J.A. Im, et al.; *Metabol. Clin. Exp.* 55, 1546 (2006)
2. T. Yasui, et al.; *Clinica. Chimica. Acta.* 386, 69 (2007)
3. J.H. Kang, et al.; *J. Korean Med. Sci.* 22, 117 (2007)
4. E.S. Kim, et al.; *Obesity (Silver Spring)* 15, 3023 (2007)
5. S. Lim, et al.; *J. Clin. Endocrinol. Metab.* 93, 2263 (2008)
6. S. Lim, et al.; *Obesity (Silver Spring)* 17, 188 (2009)

For more References please visit www.adipogen.com!

Adipogen AG

Schützenstrasse 12
CH-1410 Liestal
Switzerland
TEL: +41-61-926-60-40
FAX: +41-61-926-60-49
Email: info@adipogen.com

Adipogen, Inc.

Room 401, Venture Building B, Songdo TechnoPark,
7-50 Songdo-dong, Yeonsu-gu, Incheon,
Korea 406-840
TEL: +82-32-858-1470
FAX: +82-32-831-1470
Email: info-kr@adipogen.com