

TECO® *Adiponectin*

Total Human Adiponectin ELISA

Instructions for Use
English



Catalogue No. TE1013

always your partner

Symbol Description



Kit Instructions



Lot Number



Expiry Date



In Vitro Diagnostic



*CE Declaration of Conformity
H-CH/CA01/IVD/21123*



Storage Temperature



Manufacturer



Caution: read instructions



TE 1013



Caution: caustic



Intended use



Tests

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- Certified Management System
- EN ISO 9001
- EN ISO 13485

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Or contact our local representative in your country.

TECO® Total Human Adiponectin ELISA Kit

CONT Reagents and Materials Supplied:

Symbol	Description	Format
1	Antibody Coated Wells 12 break apart strips of 8 wells (12 x 8 in total), in a frame, Ready to use	1 plate
A	Standard A 2 ng/ml - lyophilized	1 x 0,75 ml
B	Standard B 10 ng/ml - lyophilized	1 x 0,75 ml
C	Standard C 30 ng/ml - lyophilized	1 x 0,75 ml
D	Standard D 70 ng/ml - lyophilized	1 x 0,75 ml
E	Standard E 100 ng/ml - lyophilized	1 x 0,75 ml
L	Control 1 ng/ml - lyophilized, Range as indicated on data sheet	1 x 0,5 ml
H	Control 2 ng/ml - lyophilized, Range as indicated on data sheet	1 x 0,5 ml
2	Dilution Buffer Ready for use	1 x 125 ml
3	Antibody HRP-Conjugate Ready for use	1 x 12 ml
4	TMB Substrate Ready for use	1 x 12 ml
4	Wash Buffer 20 times concentrated	1 x 50 ml
6	Stop Solution – 0,2 M H₂SO₄ 0,2 M sulfuric acid, ready for use	1 x 12 ml
7	Cover for Microtiterplate, adhesive	2 pieces
	Kit instruction	1 x

Storage

Store kit at 2–8 °C. Do not freeze. Store unused reagents at 2–8 °C.

Instructions for Use

The TECO® Total Human Adiponectin kit is a sensitive "two-site" sandwich enzyme linked immunosorbent in-vitro assay for the quantitative determination of Adiponectin in human plasma and serum.

Clinical Use

- Obesity
- Arteriosclerosis
- Energy metabolism
- Coronary diseases
- Polycystic Ovarian Syndrom

Adiponectin is a 30kDa protein, presenting 0,01 % of serum proteins. It is mainly synthesized by adipocytes, but muscle cells and hepatocytes have also the ability to synthesize Adiponectin. Until now, IGF-I is the only known natural inductor of the synthesis. It consists of a Collagen-like N-terminal and a globular C-terminal domain [1]. In vivo Adiponectin appears with different oligomers. Beside the trimer and dimer, high molecular multimers also exist [1–3]. Up to now two different receptors are known, both receptors are ubiquitous expressed, though the distribution in the tissues varies. The Adiponectin Receptor 1 (AdipoR1) is synthesized especially in muscle- and AdipoR2 in liver tissue [4].

The significance for the human organism is not completely known until now. First studies show, that Adiponectin correlates negatively with BMI and thus it could have relevance for the energy metabolism, for example through the regulation of fatty acid oxidation. Beside the correlation with BMI, Adiponectin level is associated with the Insulin-Resistance [5–7] and so, also linked with Type II Diabetes. Adiponectin is associated also with glucose- und lipometabolism [8, 9].

A special diagnostic significance for high molecular multimers, as described repeatedly, could not be proven in a comparative study of three test systems [10].

Furthermore Adiponectin is involved in inflammatory processes [11–15] and is of importance for the appearance of arteriosclerosis [4, 5, 16] and coronaritis [17, 18]. In consequence the determination of Adiponectin level in plasma could be useful to estimate the risk of coronary disease [19, 20]. Beside this, Adiponectin influences further physiological processes as for example the angiogenesis [21, 22].

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Assay Principle

The TECO® assay kit for Adiponectin is a so-called Sandwich-Assay using two specific and high affinity monoclonal antibodies. The Adiponectin in the samples binds to the first antibody coated on the microtiter plate. In the following step the second specific anti-Adiponectin-Antibody binds in turn to the immobilised Adiponectin. The second antibody is biotinylated and will be incubated in a mixture with a Streptavidin-Peroxidase-Enzyme Conjugate. In the closing substrate reaction the turn of the colour will be catalysed quantitatively depending on the Adiponectin-level of the samples.

Materials Required and not Supplied

- Pipettes capable of dispensing 5 µl, 40 µl, 80 µL, 100 µl, 320 µl, 350 µl, 500 µl, 560 µl and 750 µl
- Graduated cylinders for reconstituting or diluting reagents
- Manual Aspiration System and multi-channel pipette or automatic washer
- Aqua dest
- Vortex mixer
- ELISA plate reader suitable for 96 well formats and capable of measuring at 450 and 405 nm (Reference: 590–650 nm).
- ELISA plate shaker (≥ 400 rpm) (orbital shaker)
- Software package for data generation and analysis

Warnings and Precautions

This kit is intended for in vitro use by professional persons only.

Follow the instructions carefully.

Observe expiration dates stated on the labels and the specified stability for reconstituted reagents. Refer to "Materials Safety Data Sheet" for more detailed safety information.

Material of human origin used in the preparation of this kit has been tested and found non reactive for HIV-1 and HIV-2 as well as for HCV antibodies and HbsAg but should, nonetheless, be handled as potentially infectious.

TECOmedical AG is not liable for loss or harm caused by non-observance of the Kit instructions.

1. For in vitro diagnostic use.
2. Treat all specimen samples as potentially biohazardous material. Follow General Precautions when handling contents of this kit and any patient samples.
3. Disposal of containers and unused contents should be done in accordance with federal and local regulatory requirements.
4. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5. Store assay reagents as indicated.
6. Do not use coated strips if pouch is punctured.
7. Test each sample in duplicate.
8. Use of multichannel pipettes or repeat pipettors is recommended to ensure the timely delivery of liquids.
9. a) 0,2 M sulfuric acid is caustic and can cause severe burns.
b) handle TMB with care.
Do not ingest. Avoid contact with skin, eyes, or clothing. If contact is made, wash with water. If ingested, call a physician.
10. As preservative is used (0,01 %) 2-Methyl-4-isothiazolin-3-one solution and (0,01 %) 5-chloro-2-methyl 2H isothiazol-3-one / 2-methyl-2H-isothiazol-3-one solution for the antibody, dilution buffer and washing buffer.
Do not ingest. Avoid contact with skin, eyes, or clothing. If contact is made, wash with water. If ingested, call a physician.

Preparation of Reagents

1 Microtiterplate coated with an anti-human Adiponectin Antibody

12 break apart strips of 8 wells (96 in total) in a frame and sealed in a foil bag. Fit strip wells firmly into the frame. After opening, immediately return any unused wells to the original foil package and seal. Store at 2–8 °C until expiration date.

A Standards

till
E 5 vials of lyophilized Standard containing native Adiponectin (2, 10, 30, 70 and 100 ng/ml). Reconstitute each Standard with 750 µl of Dilution Buffer. Keep reconstituted reagents at room temperature for 15 minutes and then mix them gently (no foam!) with a Vortex. After reconstitution, the Standards are stable 2 months at -20 °C. Only one freeze/thaw cycle or keep in aliquotes. Store lyophilized at 2–8 °C.

L Control 1

1 vial of lyophilized control (human serum). Reconstitute with 500 µl of Dilution Buffer. Keep reconstituted reagents at room temperature for 15 minutes and then mix them gently (no foam!) with a Vortex. After reconstitution, the control serum is stable 2 months at -20 °C. Only one freeze/thaw cycle or keep in aliquotes. For the exact value, refer to data sheet. Store lyophilized at 2–8 °C.

H Control 2

1 vial of lyophilised control (human serum). Reconstitute with 500 µl of Dilution Buffer. Keep reconstituted reagents at room temperature for 15 minutes and then mix them gently (no foam!) with a Vortex. After reconstitution, the control serum is stable 2 months at -20 °C. Only one freeze/thaw cycle or keep in aliquotes. For the exact value, refer to data sheet. Store lyophilized at 2–8 °C.

2 Dilution Buffer

1 vial of 125 ml, ready for use. Possible precipitation in the Buffer, resolve before using by mixing and/or warming. Store at 2–8 °C until expiration date.

3 Antibody-HRP Conjugate

1 vial of 12 ml, ready for use. Anti-human adiponectin conjugated to horseradish peroxidase. Store at 2–8 °C until expiration date.

4 TMB Substrate

1 vial of 12 ml stabilized H₂O₂ Tetramethylbenzidine. Ready for use. Store at 2–8 °C until expiration date.

4 Wash Buffer

1 vial of 50 ml buffer. Possible precipitation in the Buffer, resolve before using by mixing and/or warming Bring the vial content to 1000 ml with distilled water. The diluted washing solution is stable for 4 weeks at 2–8 °C. Store undiluted at 2–8 °C until expiration date.

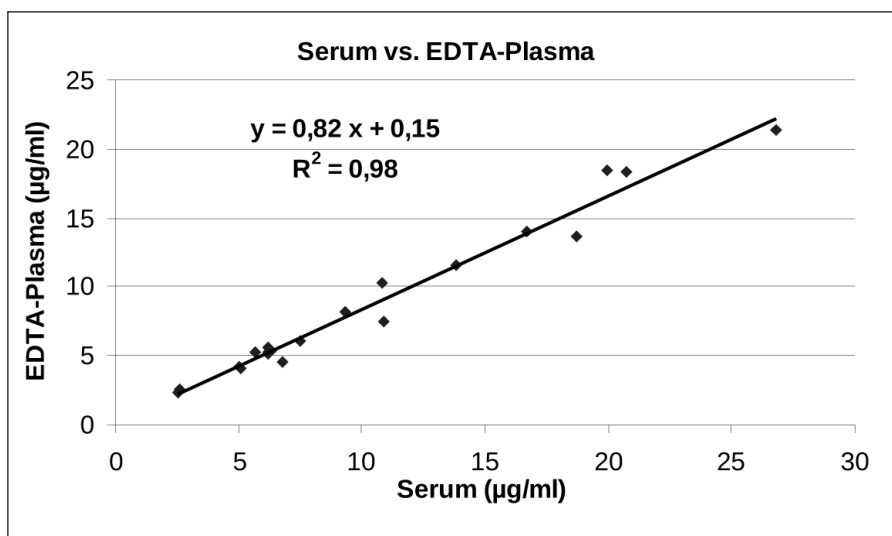
6 Stop Solution – 0,2 M H₂SO₄
1 vial of 12 ml of 0,2 M H₂SO₄. Ready for use. Store at 2–8 °C until expiration date.

7 Cover for Microtiterplate
2 pieces, adhesive.

Preparation and Stability of Serum Samples

Sample Type

Fasting blood samples recommended. Human Serum or Plasma, Breast milk, Urine, CSF and cell culture. Similar values were found in Serum and Heparin Plasma, but in EDTA and Citrate Plasma, sample values were 18 % lower.



Adiponectin: Serum vs. EDTA Plasma – 20 Samples in 5 different assay runs

Stability

- Maximum 2 days at room temperature
- Maximum 2 years at -20 °C
- Maximum 5 freeze/thaw cycles

Note

Samples have to be diluted in Dilution Buffer.

Most of the time (for serum or plasma samples, and if no extreme values are expected) a dilution of 1:200 to 1:1600 with Dilution Buffer should be suitable. According to expected Adiponectin levels the dilution can be higher or lower. The Adiponectin concentration may be completely different in body fluids of human origin other than serum or cell culture supernatants.

In general a dilution factor of 1:300 for serum or plasma samples is suitable for clinical diagnostic.

Suggestion for dilution protocol:

- **One step dilution 1:300:**
dilute **5 µl** sample in **1,5 ml** Dilution Buffer

- **Two steps dilution 1:300:**
 1. Dilute 1:15 by adding **40 µl** Serum or Plasma to **560 µl** Dilution Buffer (application of a multi-stepper is recommended in larger series).
 2. Then add **20 µl** of the thoroughly mixed first dilution to **380 µl** Dilution Buffer.
After mixing, use **2×100 µl** from this diluted sample in the assay.

Recommended dilution of samples:

- Serum / Plasma: 1:300 or higher
- Breastmilk: 1:2 to 1:10
- Urine: 1:2 to 1:10
- Saliva: 1:2 to 1:10
- CSF: 1:2 to 1:10
- Cell culture: 1:5 to 1:200

Assay Procedure

All determinations (Standards, diluted Serum Controls and diluted Samples) should be assayed in duplicate. When performing the assay, the Standards, Control Sera and Samples should be pipetted as fast as possible (<15 minutes).

To avoid distortions due to differences in incubation times, HRP Conjugate, Substrate Solution and Stop solution should be added to the plate in the same order and with the same time interval as the samples.

Allow all reagents to stand at room temperature (20–25 °C) for at least 30 minutes. After reconstitution, keep the reagents at room temperature for 15 minutes and then mix gently before use.

1. Prepare the frame and the required number of coated strips **1**.
2. Allocate the wells of the Microtiter plate for Standards, Controls and Samples.

Dilute samples, see page 11 and controls (1:300).

3. Pipette 100 µl Dilution Buffer **2** in duplicate into wells (Blank).
4. Pipette 100 µl of each Standards (**A** till **E**), diluted Control sera (**L** and **H**) and samples (see dilution protocol) into the corresponding wells.
5. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (20–25 °C) on a plate shaker (≥400–500 rpm)
6. After incubation, aspirate the wells by using a plate washer or manually decant by inverting the plate. Wash the wells 3x with 350 ml diluted washing buffer (15 seconds incubation per cycle). After the last wash cycle tap the inverted wells gently on a dry absorbent surface to remove excess wash solution.
7. Pipette 100 µl of the Antibody HRP Conjugate **3** in each well.
8. Cover the wells with sealing tape and incubate the plate for 30 minutes at room temperature (20–25 °C) on a plate shaker (≥400–500 rpm).
9. Wash the wells 3 times with Washing Buffer as described in step 6.
10. Pipette 100 µl of the TMB Substrate Solution **4** in each well.
11. Incubate the plate for 15 minutes, in the dark, at room temperature (20–25 °C).
12. Add 100 µl of Stop Solution **6** in each well.
13. Measure the colour reaction within 30 minutes at 450 nm (reference filter between 590–650 nm). With strong color reaction e. g. >3 OD also measure at 405 nm.

Protocols for the different automatic ELISA systems are available.

Result Analysis

For the evaluation of the assay, the absorbance values of the blank should be below 0,25 and the absorbance values of Standard **E** should exceed 1,0.

Samples, having higher absorbance values than Standard **E** , should be tested again with a higher dilution.

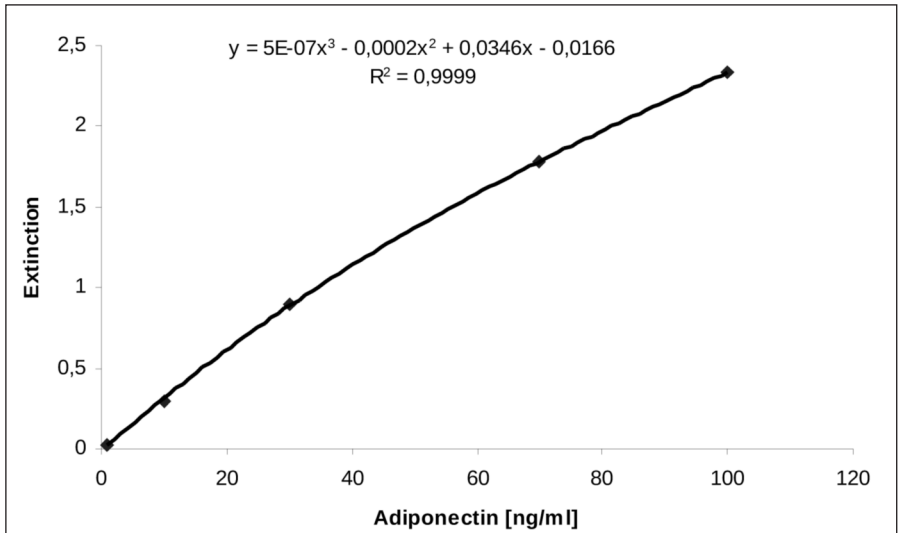
A Standard curve can be established by plotting Standard concentration on the x-axis (linear scale) against the absorbance of the Standards on the y-axis (linear scale). The Adiponectin concentrations in diluted patient sera can then be read off the Standard curve. A 4-parameter curve fit should be used for automatic data reduction. Adiponectin concentration of samples and controls will be obtained by multiplying the value read off the Standard curve by the dilution factor.

Typical Results 450 nm

(Example only. Not for use in calculation of actual results)

Standards	Absorption at 450 nm	ng/ml
A	0,06	2
B	0,33	10
C	0,89	30
D	1,77	70
E	2,50	100
L-Control 1	0,46	*4,21 µg /ml
H-Control 2	1,01	*10,6 µg /ml

* corrected by dilution factor 1:300



For each assay, the results of the controls must be within the target range indicated for every lot. The QC protocol with target ranges is provided with the kit. If control values are not within the limits of the target range, the assay results should be considered questionable and the samples should be tested again.

Reference Values

The expected values for serum Adiponectin, which were determined with the TECO® ELISA Kit in healthy donors are given below. These data show significant correlation between Adiponectin-Serum values and age as well as gender of the healthy subjects. In turn the correlation between the respective BMI seems to be less significant. Very high Adiponectin values were found in the samples of neonatal cord blood.

Expected values for adults, gender specific

Gender	n	Mean (µg/ml)	Median (µg/ml)	SD	5 th Percentile (µg/ml)	95 th Percentile (µg/ml)
Women	101	10,2	9,1	4,6	4,0	19,4
Men	125	6,8	6,1	4,1	2,0	13,9
All	226	8,3	7,5	4,6	2,4	19,3

Expected values for children and juveniles, gender specific

Gender	n	Mean (µg/ml)	Median (µg/ml)	SD	5 th Percentile (µg/ml)	95 th Percentile (µg/ml)
Girls	131	8,71	8,18	4,32	3,05	15,6
Boys	134	8,97	8,12	5,13	3,36	18,6
All	265	8,84	8,18	4,74	3,33	16,5

Expected values, age specific

Age (in Years)	n	Mean (µg/ml)	Median (µg/ml)	5 th Percentile (µg/ml)	95 th Percentile (µg/ml)
< 8	46	12,82	11,70	2,33	26,50
8–10	40	8,00	8,09	3,96	14,90
10–12	55	8,02	7,14	3,36	13,80
12–14	26	8,21	7,54	4,50	13,20
14–16	59	8,12	8,09	3,67	13,70
16–20	41	7,97	7,79	2,74	13,30
all	267	8,88	8,18	3,33	16,70

20–30	47	6,72	6,38	2,50	12,25
30–40	38	7,38	6,69	1,98	19,29
40–50	55	8,42	8,20	2,41	17,85
50–60	47	9,61	8,55	2,15	19,85
>60	32	6,52	8,57	3,00	21,10
all	226	8,33	7,50	2,41	19,29

Expected values, age and gender specific related to BMI

Female			Adiponectin (µg/ml)			
Age (in Years)	n	BMI Mean ± SD	Mean ± SD	Median	Percentile 25 th – 75 th	Min. – Max.
Newborn Cord blood	19		29,80±12,49	26,1	19,5–35,2	16,9–61,4
<4	9	15,73±0,79	14,43±7,76	11,2	8,2–21,8	2,3–26,7
4–8	11	16,01±1,94	8,46±4,73	9,3	2,9–12,1	1,4–15,6
8–10	22	17,58±3,84	7,92±3,00	8,2	5,2–10,0	3,6–15,1
10–12	33	17,83±1,86	7,66±4,59	6,6	5,0–8,8	3,1–20,9
12–14	11	19,85±2,31	8,22±5,64	7,5	6,5–9,2	4,9–13,2
14–16	27	19,91±1,72	8,83±9,25	8,9	5,2–11,8	2,6–17,7
16–20	18	21,64±2,64	9,00±3,22	8,7	6,9–11,2	2,7–14,0
20–30	24	23,12±5,01	7,39±3,35	7,3	5,7–9,0	3,4–17,8
30–40	17	23,20±2,86	9,19±3,89	8,6	7,2–10,4	3,6–19,3
40–50	26	24,50±4,11	9,93±3,59	9,5	7,5–11,6	4,4–19,6
50–60	21	24,61±3,31	11,50±5,49	10,0	8,0–15,9	2,0–23,1
>60	8	24,63±1,89	15,60±4,64	15,3	11,4–18,2	11,2–24,1

Male			Adiponectin (µg/ml)			
Age (in Years)	n	BMI Mean ± SD	Mean ± SD	Median	Percentile 25 th – 75 th	Min. – Max.
Newborn Cord blood	10		27,80±7,68	26,7	22,2–31,0	15,6–40,6
<4	14	16,17±1,81	16,57±6,55	14,3	11,6–21,2	5,8–40,3
4–8	12	15,69±1,05	11,24±5,43	9,7	8,9–15,9	3,5–18,6
8–10	18	16,45±1,76	8,11±2,93	7,6	6,2–9,1	5,0–15,4
10–12	21	18,34±2,18	8,43±3,91	7,8	5,2–10,9	3,4–20,2
12–14	14	18,61±2,11	7,59±2,86	7,1	6,0–10,3	2,4–12,2
14–16	32	19,86±2,00	7,53±2,52	7,4	5,1–9,3	3,8–15,4
16–20	23	22,03±2,42	7,16±3,53	6,9	4,2–9,6	2,0–13,9
20–30	23	23,43±2,48	5,44±2,29	5,8	4,0–6,9	1,3–10,3
30–40	21	23,33±2,72	5,92±4,60	4,4	2,7–6,7	1,9–20,6
40–50	22	23,79±2,41	6,13±2,92	5,5	3,8–8,3	2,1–11,6
50–60	23	26,68±2,77	7,45±4,50	6,7	5,0–8,8	1,4–19,6
>60	24	25,72±2,12	7,48±3,92	7,6	4,6–9,2	3,0–21,1

BMI=Body Mass Index (kg/m²)

Test Performance

Calibration

The Standards in the kit are prepared from native Adiponectin (Human Serum), and quantified against a recombinant protein.

Precision

(Inter assay)

Sample	N	Mean value (µg/ml)	Standard deviation (µg/ml)	CV (%)
Sample 1	22	4,76	0,28	5,88
Sample 2	25	5,22	0,35	6,72
Sample 3	25	5,62	0,32	5,70
Sample 4	25	11,57	0,68	5,90

(Intra assay)

Sample	N	Mean value (µg/ml)	Standard deviation (µg/ml)	CV (%)
Sample 1	16	5,87	0,138	2,35
Sample 2	16	12,19	0,377	3,10
Sample 3	6	14,36	0,668	4,66

Detection Limit

The kit zero Standard was assayed 16 times and the mean and standard deviation were calculated. The lower detection limit at +2 standard deviations is 0.6 ng/ml.

Recovery Test

The recovery of recombinant Adiponectin yielded in a serum matrix on average 110 %.

Adiponectin (ng/ml)	9,38	18,75	37,5	75
% Recovery	109	116	101	113

Dilution Test

Dilution	Sample 1 (recalculated, µg/ml)	Dilution	Sample 2 (recalculated, µg/ml)
1:200	12,49	1:200	11,58
1:400	11,92	1:400	11,74
1:600	10,80	1:600	11,41
1:800	11,17	1:800	11,35
1:1000	12,06	1:1000	10,58
1:1200	11,64	1:1200	10,96
1:1400	10,86	1:1400	11,18
1:1600	10,75	1:1600	10,61
Mean/1SD/CV %	11,46/0,66/5,8	Mean/1SD/CV %	11,18/0,43/3,8

Interference

Serum samples have been spiked with different concentrations of possibly interfering substances and the amount of adiponectin was measured and compared with the adiponectin concentration in the same sample without any spiking. None of the tested substances interfered significantly with adiponectin measurement.

%	Triglyceride 100 mg/ml	Bilirubin 100µg/ml	Hemoglobin 100µg/ml
Sample 1	95	97	90
Sample 2	90	93	97
Sample 3	95	94	93

Cross-reactivity

No cross reactivity with the following species:

Horse, Cow, Chicken, Rabbit, Dog, Guinea pig, Sheep, Mouse, Goat, Donkey, Rat, Cat.

Remark

The data quoted in this instruction should be used for guidance only. It is recommended that each laboratory includes its own panel of control samples in the assay. In order to follow GLP guidelines, each laboratory should establish its own normal and pathological ranges for Adiponectin levels.

TECO® Total Human Adiponectin

Assay Procedure – Quick Guide

- Bring samples and reagents to room temperature. Mix the samples well.
- Dilute samples and controls.
- Standards **A** till **E**: Reconstitute each vial with 750 µl Dilution Buffer **2**.
- Controls **H** and **L**: Reconstitute each vial with 500 µl Dilution Buffer **2**.
- Washing Buffer **4**: Dilute 1:20 with Aqua dest.

Prepare the required number of Assay Strips **1**

Pipette **100 µl** Dilution Buffer **2** into wells (Blank)

Pipette **100 µl** Standards **A** till **E**, diluted Controls **L** and **H** and diluted Samples

Cover the plate and incubate **60 min** at 20–25 °C on a shaker ≥400–500 rpm

Aspirate and wash **3 x** with **350 µl** Wash Buffer, aspirate and tap the inverted wells gently on a clean dry absorbent surface

Add **100 µl** Antibody HRP Conjugate **3** into each well

Cover the plate and incubate **30 min** at 20–25 °C on a shaker ≥400–500 rpm

Aspirate and wash **3 x** with **350 µl** Washing Buffer, aspirate and tap the inverted wells gently on a clean dry absorbent surface

Add **100 µl** TMB Substrate **4** into each well

Incubate **15 min** at 20–25 °C **in the dark**

Add **100 µl** Stop Solution **6** into each well

Measure the absorbance at 450 nm within 30 minutes

Quantification software, 4-parameter fit:

$$y = (A-D)/(1+(x/C)^B)+D$$

Reference measurement should be performed at 590–650 nm



Please read Kit instruction before using the Quick Guide