



Adiponectin Elisa

KAPME09

PRODUCT INFORMATION AND MANUAL

Adiponectin

ELISA

KAPME09

**Enzymeimmunoassay for quantitative Determination of
human Adiponectin in serum and plasma**



DIAsource Adiponectin ELISA KAPME09

- is suited for Adiponectin determination in **Serum** and **Plasma** samples,
- age- and sex specific **reference values** are available
- is extremely sensitive (less than **0.6 ng/ml**)
- is **fast**: incubation time a total of 1 hour and 45 minutes
- Single Standards with **2, 10, 30, 70, 100 ng/ml** human Adiponectin are provided in the Kit
- 2 Control Sera are provided for quality control purposes according GLP
- is calibrated with native **Adiponectin** and correlated to a commercialised radioimmunoassay
- uses **high affinity monoclonal antibodies** against human Adiponectin
- Microtiter plates are separately breakapart

Intended Use

Measurement of human Adiponectin in human serum and plasma samples.

Introduction

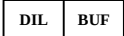
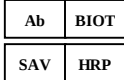
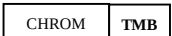
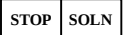
Adiponectin is a 30kDa protein which percentage in serum proteins is 0.01%. It is mainly synthesized by Adipocytes, but also muscle cells and hepatocytes have the ability to synthesize Adiponectin. Until now, IGF-I is the only known natural inducer of the synthesis. It consists of a Collagen-like N-terminal and a globular C-terminal domain [1]. In vivo Adiponectin appears with different oligomers. Beside the trimer and dimer also high molecular multimers exist [1-3]. Up to now two different receptors are known, both receptors are ubiquitarily expressed, though the distribution in the tissues varies. The Adiponectin Receptor 1 (AdipoR1) is especially in muscle- and AdipoR2 in liver tissue synthesized [4].

The significance for the human organism is not clear 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- and lipometabolism [8, 9].

The formerly proposed diagnostic value of the high molecular weight form of adiponectin was not verified using a commercially available testsystem for the determination of HMW adiponectin [10]. Blueher et al. clearly demonstrate that regarding the diagnosis of insulin resistance, measured by whole body glucose uptake below 40 $\mu\text{mol/kg}\cdot\text{min}$, total adiponectin as determined with the DIAsource KAPME09, is with an area of 0.92 under the receiver-operating curve, of greater diagnostic value [10].

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

Reagents Provided

1)		Microtiter plate , ready for use: Microtiter plate with 96 wells, dived up in 12 stripes of 8 wells (separately breakapart), coated with anti-human Adiponectin antibody.
2)		Cal N , lyophilised, contain native Adiponectin. Calibrator values are between 2-100 ng/ml (2, 10, 30, 70 and 100 ng/ml) Adiponectin and have to be reconstituted in 750 μl (each) Dilution Buffer . 100 μl per well are used in the assay. If the calibrators are required for more than one assay process we recommend to store the reconstituted calibrators frozen at -20°C. Attention: Calibrators should be thawed only once – where required please store aliquoted in adequate volumes.
3)		Dilution buffer , 125 ml , ready for use, please use for the reconstitution of Calibrators, Control Sera 1 & 2 and for the serum dilution.
4)		Control Serum 1 & 2 , each 500 μl lyophilised: Contains human Serum and has to be reconstituted in 500 μl Dilution Buffer . The Adiponectin target value concentration and the respective range is on the vial label. The dilution of the Control Sera 1&2 should be according to the dilution of the respective samples, the target value concentration should be obtained by multiplication with the respective dilution factor .
5)		Antibody-Conjugate , 12 ml , ready for use , contains a mixture of biotinylated anti-Adiponectin antibody and HRP (Horseradish Peroxidase)-labelled Streptavidin. Use 100 μl /well in the assay.
6)		Washing Buffer , 50 ml , 20 X concentrated solution. Washing Buffer has to be diluted 1:20 with distilled or demineralised water before use (e.g. add the complete contents of the flask (50 ml) into a graduated flask and fill up with A.dest. to 1000 ml). Attention: After dilution the Washing Buffer is only limited stable, dilute only according to requirements.
7)		Chromogenic Substrate , 12 ml , ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2 Tetramethylbenzidine.
8)		Stopping Solution , 12 ml , ready for use, 0.2 M sulphuric acid, Caution acid!
9)		Sealing tape for covering of the microtiter plate, 2 x, adhesive.

Materials Required but not provided

Precision pipettes (100 and 200µl) Micropipettes and multichannel pipettes with disposable plastic tips
Distilled or Deionized water for dilution of the Washing Buffer
Vortex-mixer
Device to aspirate the standards and the samples from the wells (recommended because of the potential danger of infection by human samples)
Timer (120 min. range)
Reservoirs (disposable)
Plate washer and plate shaker (recommended)
Calibrated Micro plate reader ("ELISA-Reader") with filter for 450 and 620nm (or ≥590 nm)
Foil welding device for laminate bags (recommended)

WARNINGS AND PRECAUTIONS**For in-vitro diagnostic use only.**

Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

Before use, all kit components should be brought to **room temperature at 20 - 25°C**. Precipitates in buffers should be dissolved before use by thorough mixing and warming.

Do not mix reagents of different lots. Do not use expired reagents.

The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.

Caution: This kit contains material of human and/or animal origin. Source human sera for the Control Sera provided in this kit were tested by FDA recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibody. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.

ProClin 950

Following components contain < 0.01% 2-Methyl-4-isothiazolin-3-one solution as preservative : calibrators 1-5, Antibody-conjugate, dilution buffer

R34	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36/37	Wear suitable protective clothing and gloves
S45	In case of accident or if you feel unwell seek medical advice

Kathon CG

Following components contain < 0.01%(w/w) 5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-isothiazol-3-one as preservative: calibrators 1-5, Antibody-conjugate, dilution buffer, washing buffer

R36/38	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice S28.1 S28.1
	After contact with skin, wash immediately with plenty of water

Stop solution contains 0.2 M Sulfuric Acid (H₂SO₄)

R36/38	Irritating to eyes and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves.

Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step. Use separate pipette tips for each sample, control and reagent to avoid cross contamination. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

TMB-Substrate contains 3,3',5,5' Tetramethylbenzidine. Store and Incubate in the dark.

R20/21/R22	Harmful by inhalation, in contact with skin and if swallowed
R36/37/38	Irritating to eyes, respiratory system and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves

General first aid procedures:

Skin contact: Wash affected area thoroughly with water. Discard contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.

Ingestion: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

Method

The DIAsource ELISA for Adiponectin KAPME09 is a so-called Sandwich-Assay using two specific and high affinity 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 applied 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.

Specimen

Serum and plasma samples are applicable. In EDTA- and Citrate Plasma-samples levels were found approx. 18% lower. Adiponectin can also be measured in urine, breast milk and cellculture media by this testsystem.

The blood sample for serum preparation should be obtained according to standardized venipuncture procedure. The samples should be stored without anticoagulation reagents. Hemolytic reactions have to be avoided. The blood has to be allowed to clot and after complete clotting, serum is separated by centrifugation.

Storage of the samples

Storage at RT max. 2 days

Storage at -20°C max. 2 years

More than five freeze/thaw cycles are not possible.

Sample Preparation

Samples have to be diluted in Dilution Buffer. The excellent linearity of this testsystem allows sample dilution of 1:200 to 1:1600.

For clinical purposes we recommend a standard dilution of 1:310.

Suggestion for dilution protocol:

Add for example 300 µl Dilution Buffer in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series) and add 10 µl Serum- or Plasma (dilution: 1:31). Add **900µl** Dilution Buffer in an other PE-/PP-tube and **100µl** of the thoroughly mixed first dilution. After mixing, use **2×100 µl** from this **1:310** diluted sample in the assay.

If you have the necessary technical equipment a one-step dilution of 1:301 is possible: Ad 5µL to 1.5mL dilution buffer .

Technical Recommendations

The assay has to be conducted strictly according the test protocol herein.

Reagents with different lot numbers cannot be mixed. The microtiterplate and reagents are stable until the indicated expiry if stored unopened and protected from sunlight at 2 – 8°C.

Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming.

Incubation at room temperature means: 20-25°C

Calibrators and Controls

For the reconstitution of the lyophilised components (Calibrators 1-5 and Control Sera 1&2) the kit Dilution Buffer has to be used. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam!) with a Vortex mixer.

The reconstituted standard and controls can be stored for 2 month at -20°C. Repeated freeze/thaw cycles have to be avoided.

Washing Buffer

The required volume of washing buffer is prepared by 1:20 dilution of the provided 20fold concentrate with deionised water. The diluted Washing Buffer is stable for 4 weeks at 2-8°C. It has to be at room temperature for usage!

Microtiterplate

Unused microtiterplate stripes have to be stored airtight together with the desiccant bag at 2-8°C. The labelled expiry is not influenced in case of proper storage.

Assay Procedure

All determinations (Calibrators, Control Sera 1 & 2 and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

When performing the assay, the Calibrators, Control Sera and the samples should be pipette as fast as possible (e.g., <15 minutes).

All incubations have to be conducted at room temperature (20-25°C)

To avoid distortions due to differences in incubation times, Antibody-Conjugate as well as the following Substrate Solution should be added to the plate in the same order and in the same time interval as the samples. Stop Solution should be added to the plate in the same order as the Chromogenic substrate.

- 1) Add **100 µl** Dilution Buffer in the first 2 wells. Subsequently add 100 µl Calibrator or 100 µl of diluted Control Sera or diluted samples.
- 2) Cover the wells with sealing tape and incubate the plate for **1 hour at room temperature** (if possible, shake at ≥350 rpm)
- 3) After incubation aspirate the contents of the wells and wash the wells 3 times **250 µl Washing Buffer** / well. The washing buffer should incubate for at least for 15 seconds/cycle.
- 4) Following the last aspirating step pipette **100 µl** of the **Antibody-Conjugate** in each well.
- 5) Cover the wells with sealing tape and incubate the plate for **30 Minutes at room temperature** (if possible shake ≥350 rpm).

- 6) After incubation wash the wells 3 times with Washing Buffer as described in step 3
- 7) Pipette **100 µl of the Chromogenic Substrate** Solution in each well.
- 8) Incubate the plate for **15 minutes in the dark at room temperature (20 - 25°C)**.
- 9) Stop the reaction by adding **100 µl of Stopping Solution**.
- 10) Measure the colour reaction within 30 minutes at 450nm (reference filter ≥590 nm).

Calculation of Results

Establishing the Calibration Curve

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be below 0.25, these of calibrator 5 should exceed 1.0.

Samples, which yield higher absorbance values than calibrator 5 are beyond the calibration curve, for reliable determinations these samples should be tested again after a higher dilution.

Calibrators are provided in the following concentrations (use the concentration unit as preferred):

Calibrator	1	2	3	4	5
ng/ml	2	10	30	70	100
µg/ml	0.002	0.01	0.03	0.07	0.1

- 1) Calculate the **mean absorbance (MA)** value for the blank from the duplicated determination (well A1/A2).
- 2) Subtract the mean absorbance (MA) of the blank from the mean absorbances of all other values.
- 3) Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis on semi-log paper (lin-log).
- 4) Recommendation: Calculation of the calibration curve should be done by using a computer program. **Non-linear regression**, a higher-grade polynomial or four parametric logistic (4-PL) curve fit are in general suited for the evaluation.
- 5) The Adiponectin concentration of the diluted sample or the diluted control sera 1&2 in ng/ml (or µg/ml according the chosen unit for the standards) is calculated in this way, the Adiponectin concentration of the **undiluted sample** and of 1 & 2 is calculated **by multiplication** with the respective dilution factor.

The exemplary shown calibration curve in Fig.1 **cannot** be used for calculation of your test results. You have to establish a calibration curve for each test you conduct!

Exemplary calculation of the adiponectin concentration of a 1:310 diluted sample:

Measured extinction of your sample 0.39
 Measured extinction of the blank 0.04

Your measurement programm will calculate the adiponectin concentration of the diluted sample automatically by using the difference of sample and blank for the calculation. You only have to determine the most suitable curve fit (here: polynomial 3rd degree).

In this exemplary case the following equation is solved by the programm to calculate the adiponectin concentration in the sample:

$$\begin{array}{lcl} 0.35 & = & 5 \times 10^{-7} x^3 - 0.0002x^2 + 0.0346x - 0.0166 \\ 11.13 & = & x \end{array}$$

if the dilution factor (**1:310**) is taken into account the adiponectin concentration of the undiluted sample is

$$11.13 \times 310 = 3450.3 \text{ ng/mL} = 3.45 \text{ µg/mL}$$

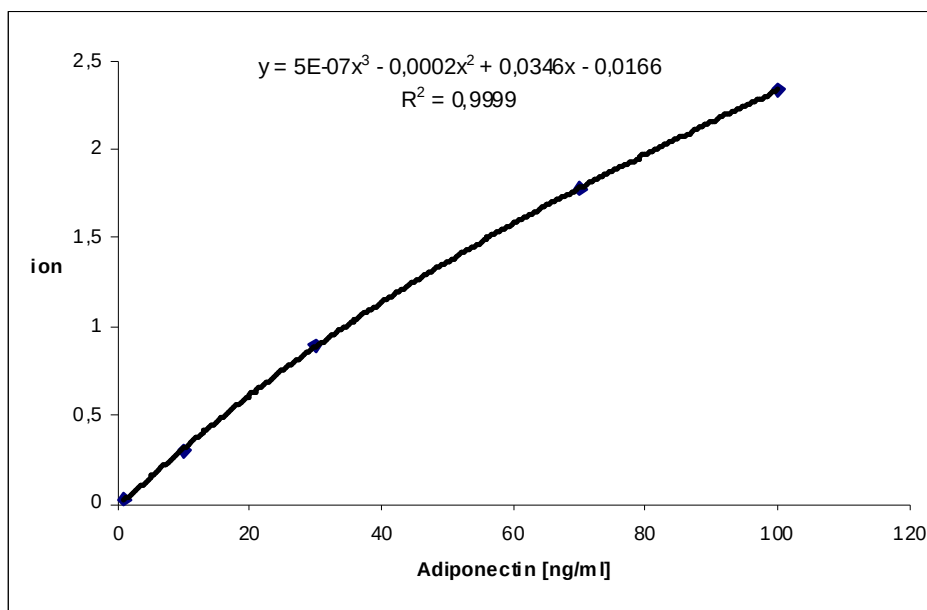


Fig. 1 Exemplary Calibration Curve with a polynomial 3rd degree as curve fit.

Performance Characteristics

Calibrators

The Calibrators of the ELISA KAPME09 are prepared from **native Adiponectin** (Human Serum) in concentrations of 2, 10, 30, 70 and 100 ng/ml. The native Adiponectin was quantified with a recombinant protein and with a commercialised radio immunoassay (Linco Corp.) for Adiponectin.

Sensitivity

The analytical sensitivity of the ELISA KAPME09 yields < 0.6 ng/ml (equal to < 0.06 ng per well; as 2xSD of zero standard in 16fold determination).

Specificity

Serum of the cited species was diluted (1:505) and used as sample in this assay system. No cross reactivity was detected:

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

Interference

Interference of physiological appearing substance with the adiponectin measurement was investigated. Serum samples have been enriched 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 enrichment. In Table 1 the relative results are shown. None of the tested substances interfered significantly with adiponectin measurement.

Table 1: %-Recovery compared to non-enriched serum

%	Triglyceride 100 mg/mL	Bilirubin 100µg/mL	Hemolysat 100µg/mL
Serum 1	95	97	90
Serum 2	90	93	97
Serum 3	95	94	93

Recovery

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

Table 2: Recovery of recombinant Adiponectin

Adiponectin [ng/mL]	9.38	18.75	37.5	75
% Recovery	109	116	101	113

Reproducibility and Precision

The inter- and intra assay coefficients of variability are below 6.7% and 4.7%, respectively. Exemplary determinations are shown in table 3 and 4.

Table 3: Inter-Assay-Variation

	Number of single determinations	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

Table 4: Intra-Assay-Variation

	Number of determinations	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

Linearity

The DIAsource Adiponectin ELISA KAPME09 is over a very wide range dilution authentic, the linearity of serum dilutions is over a very wide range excellent (s.Tab.5).

Table 5: Linearity

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	1:100	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
AV / 1SD / CV%	11.46 / 0.66 / 5.8	AV / 1SD / CV%	11.18/ 0.43 / 3.8

AV = Average Value , SD = Standard Deviation

Expected Reference Values

The expected values for serum adiponectin, which were determined with the DIAsource ELISA KAPME09 in healthy donors and analysed by Prof. Dr. J. Kratzsch, Department of Laboratory Medicine, University Hospital Leipzig, are given below (Tab. 6). Several different statistical analyses were performed to adapt for certain individual demands. The best suited data can be chosen respectively for interpretation of the own measurements.

These data show significant correlation between Adiponectin-Serum values and age as well as gender of the probands, in turn the correlation between the respective BMI seems to be less significant. In the samples of neonatal cord blood very high values were found.

Table 6a: Expected values for adults, gender specific mean as well as median, 5. and 95. percentile are given.

Sex	number	Mean [µg/ml]	Median [µg/ml]	Standard Deviation	5 th Percentile [µg/ml]	95 th Percentile [µg/ml]
Female	101	10.2	9.1	4.6	4.0	19.4
Male	125	6.8	6.1	4.1	2.0	13.9
total	226	8.3	7.5	4.6	2.4	19.3

Table 6b: Expected values for children, gender specific mean as well as median, 5. and 95. percentile are given.

Sex	number	Mean [µg/ml]	Median [µg/ml]	Standard Deviation	5 th Percentile [µg/ml]	95 th Percentile [µg/ml]
Female	131	8.71	8.18	4.32	3.05	15.6
Male	134	8.97	8.12	5.13	3.36	18.6
total	265	8.84	8.18	4.74	3.33	16.5

Table 6c: Expected values for Adiponectin, **age specific** mean as well as median, 5. and 95. Percentile are given.

Age (in years)	number	Mean [µg/ml]	Median [µg/ml]	5 th Percentile [µg/ml]	95 th Percentile [µg/ml]
< 7.99	46	12.82	11.7	2.33	26.5
8 – 9.99	40	8	8.09	3.96	14.9
10-11.99	55	8.02	7.14	3.36	13.8
12 – 13.99	26	8.21	7.54	4.5	13.2
14 – 15.99	59	8.12	8.09	3.67	13.7
16 – 19.99	41	7.97	7.79	2.74	13.3
all	267	8.88	8.18	3.33	16.7
20 – 29.99	47	6.72	6.38	2.5	12.25
30 – 39.99	38	7.38	6.69	1.98	19.29
40 – 49.99	55	8.42	8.20	2.41	17.85
50 – 59.99	47	9.61	8.55	2.15	19.85
> 60	32	9.52	8.57	3.00	21.10
all	226	8.33	7.5	2.41	19.29

Table 6d: Expected values for Adiponectin, **age** as well as **gender specific** mean and median, BMI and 5. and 95. percentile are given.

Female			Adiponectin (µg/ml):			
Age (Years):	n:	BMI: AV ± SD	AV ± SD::	Median :	Percentile: 25.- 75.	Min. – Max.:
Newborn Cord blood	19		29.80 ± 12.49	26.1	19.5-35.2	16.9-61.4
< 3.99	9	15.73 ± 0.79	14.43 ± 7.76	11.2	8.2-21.8	2.3-26.7
4.0-7.99	11	16.01 ± 1.94	8.46 ± 4.73	9.3	2.9-12.1	1.4-15.6
8.0-9.99	22	17.58 ± 3.84	7.92 ± 3.00	8.2	5.2-10.0	3.6-15.1
10.0-11.99	33	17.83 ± 1.86	7.66 ± 4.59	6.6	5.0-8.8	3.1-20.9
12.0-13.99	11	19.85 ± 2.31	8.22 ± 5.64	7.5	6.5-9.2	4.9-13.2
14.0-15.99	27	19.91 ± 1.72	8.83 ± 9.25	8.9	5.2-11.8	2.6-17.7
16.0-19.99	18	21.64 ± 2.64	9.00 ± 3.22	8.7	6.9-11.2	2.7-14.0
20.0-29.99	24	23.12 ± 5.01	7.39 ± 3.35	7.3	5.7-9.0	3.4-17.8
30.0-39.99	17	23.20 ± 2.86	9.19 ± 3.89	8.6	7.2-10.4	3.6-19.3
40.0-49.99	26	24.50 ± 4.11	9.93 ± 3.59	9.5	7.5-11.6	4.4-19.6
50.0-59.99	21	24.61 ± 3.31	11.5 ± 5.49	10.0	8.0-15.9	2.0-23.1
>60.0	8	24.63 ± 1.89	15.6 ± 4.64	15.3	11.4-18.2	11.2-24.1

n= Number of Probands **AV**=Average Value, **BMI**=Body Mass Index (kg/m²) **SD**=Standard Deviation

Male			Adiponectin (µg/ml):			
Age (Years):	n:	BMI: AV ± SD	AV ± SD:	Median :	Percentile: 25.- 75.	Min. – Max.:
Newborn Cord blood	10		27.80 ± 7.68	26.7	22.2-31.0	15.6-40.6
< 3.99	14	16.17 ± 1.81	16.57 ± 6.55	14.3	11.6-21.2	5.8-40.3
4.0-7.99	12	15.69 ± 1.05	11.24 ± 5.43	9.7	8.9-15.9	3.5-18.6
8.0-9.99	18	16.45 ± 1.76	8.11 ± 2.93	7.6	6.2-9.1	5.00-15.4
10.0-11.99	21	18.34 ± 2.18	8.43 ± 3.91	7.8	5.2-10.9	3.4-20.2
12.0-13.99	14	18.61 ± 2.11	7.59 ± 2.86	7.1	6.0-10.3	2.4-12.2
14.0-15.99	32	19.86 ± 2.00	7.53 ± 2.52	7.4	5.1-9.3	3.8-15.4
16.0-19.99	23	22.03 ± 2.42	7.16 ± 3.53	6.9	4.2-9.6	2.0-13.9
20.0-29.99	23	23.43 ± 2.48	5.44 ± 2.29	5.8	4.0-6.9	1.3-10.3
30.0-39.99	21	23.33 ± 2.72	5.92 ± 4.60	4.4	2.7-6.7	1.9-20.6
40.0-49.99	22	23.79 ± 2.41	6.13 ± 2.92	5.5	3.8-8.3	2.1-11.6
50.0-59.99	23	26.68 ± 2.77	7.45 ± 4.50	6.7	5.0-8.8	1.4-19.6
>60.0	24	25.72 ± 2.12	7.48 ± 3.92	7.6	4.6-9.2	3.0-21.1

n= Number of Probands AV=Average Value, BMI=Body Mass Index (kg/m²) SD=Standard Deviation

Literature

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SUMMARY – DIAsource Adiponectin ELISA KAPME09

Reagent preparation:	Reconstitution:	Dilution
Calibrators 1-5	in 750 µl Dilution Buffer	
Control Sera 1 & 2	in 500 µl Dilution Buffer	1:310 with Dilution Buffer
Washing Buffer		1:20 with Aqua. dest. (e.g., add the complete contents of the flask (50 ml) into a graduated flask and fill with A.dest. to 1000 ml).

Sample Dilution: Pipette for example 300 µl Dilution Buffer in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 10 µl Serum- or Plasma (dilution: 1:31). Add **900 µl** Dilution Buffer in an other PE-/PP-tube and **100 µl** of the thoroughly mixed first dilution. After mixing, use **2×100 µl** from this **1:310** diluted sample in the assay.

Assay Procedure for Double Determination:

Pipette	Reagents	Position
100 µl	Dilution Buffer	A1/2
100 µl	Calibrator 1 (2 ng/ml)	B1/2
100 µl	Calibrator 2 (10 ng/ml)	C1/2
100 µl	Calibrator 3 (30 ng/ml)	D1/2
100 µl	Calibrator 4 (70 ng/ml)	E1/2
100 µl	Calibrator 5 (100 ng/ml)	F1/2
100 µl	Control Serum 1	G1/2
100 µl	Control Serum 2	H1/2
100 µl	Sample dilution	following wells

Cover the wells with the sealing tape.

Incubation: 1 h at RT, ≥ 350 rpm

3x 250 µl	Aspirate the contents of the wells and wash 3x with 250 µl Wash Buffer	each well
100 µl	Antibody-HRP-Conjugate	each well

Incubation: 30 min at RT, ≥350 rpm

3x 250 µl	Aspirate the contents of the wells and wash 3x with 250 µl Wash Buffer	each well
100 µl	Substrate Solution	each well

Incubation: 15 min in the Dark at RT

100 µl	Stopping Solution	each well
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Measure the absorbance within **30 min** at **450 nm** with **≥590 nm** as reference wavelength.

REF KAPME09



International Test description

Revision date : 2010-02-08

	<u>Used symbols</u>	<u>Symboles utilisés</u>
	Consult instructions for use	Consulter les instructions d'utilisation
	Storage temperature	Température de conservation
	Use by	Utiliser jusque
	Batch code	Numéro de lot
	Catalogue number	Référence de catalogue
	Control	Contrôle
	In vitro diagnostic medical device	Dispositif médical de diagnostic in vitro
	Manufacturer	Fabricant
	Contains sufficient for <n> tests	Contenu suffisant pour <n> tests
	Wash solution concentrated	Solution de lavage concentrée
	Zero calibrator	Calibrateur zéro
	Calibrator #	Calibrateur #
	Control #	Contrôle #
	Tracer	Traceur
	Tracer	Traceur
	Tracer concentrated	Traceur concentré
	Tracer concentrated	Traceur concentré
	Tubes	Tubes
	Incubation buffer	Tampon d'incubation
	Acetonitrile	Acétonitrile
	Serum	Sérum
	Specimen diluent	Diluant du spécimen
	Dilution buffer	Tampon de dilution
	Antiserum	Antisérum
	Immunoabsorbent	Immunoabsorbant
	Calibrator diluent	Diluant de calibrateur
	Reconstitution solution	Solution de reconstitution
	Polyethylene glycol	Glycol Polyéthylène
	Extraction solution	Solution d'extraction
	Elution solution	Solution d'élution
	Bond Elut Silica cartridges	Cartouches Bond Elut Silica
	Pre-treatment solution	Solution de pré-traitement
	Neutralization solution	Solution de neutralisation
	Tracer buffer	Tampon traceur
	Microtiterplate	Microplaque de titration
	HRP Conjugate	HRP Conjugué
	HRP Conjugate	HRP Conjugué
	HRP Conjugate concentrate	HRP Conjugué concentré
	HRP Conjugate concentrate	HRP Conjugué concentré
	Conjugate buffer	Tampon conjugué
	Chromogenic TMB concentrate	Chromogène TMB concentré
	Chromogenic TMB solution	Solution chromogène TMB
	Substrate buffer	Tampon substrat
	Stop solution	Solution d'arrêt
	Incubation serum	Sérum d'incubation
	Buffer	Tampon
	AP Conjugate	AP Conjugué
	Substrate PNPP	Tampon PNPP
	Biotin conjugate concentrate	Biotine conjugué concentré
	Avidine HRP concentrate	Avidine HRP concentré
	Assay buffer	Tampon de test
	Biotin conjugate	Biotine conjugué
	Specific Antibody	Anticorps spécifique
	Streptavidin HRP concentrate	Concentré streptavidine HRP
	Non-specific binding	Liant non spécifique
	2nd Antibody	Second anticorps
	Acidification Buffer	Tampon d'acidification

	<u>Gebruikte symbolen</u>	<u>Gebrauchte Symbolen</u>
	Raadpleeg de gebruiksaanwijzing	Gebrauchsanweisung beachten
	Bewaartemperatuur	Lagern bei
	Houdbaar tot	Verwendbar bis
	Lotnummer	Chargenbezeichnung
	Catalogusnummer	Bestellnummer
	Controle	Kontrolle
	Medisch hulpmiddel voor in-vitro diagnostiek	In Vitro Diagnostikum
	Fabrikant	Hersteller
	Inhoud voldoende voor <n> testen	Ausreichend für <n> Ansätze
	Wasoplossing, geconcentreerd	Waschlösung-Konzentrat
	Nulkalibrator	Null kalibrator
	Kalibrator #	Kalibrator #
	Controle #	Kontrolle #
	Tracer	Tracer
	Tracer	Tracer
	Tracer geconcentreerd	Tracer Konzentrat
	Tracer geconcentreerd	Tracer Konzentrat
	Buisjes	Röhrchen
	Incubatiebuffer	Inkubationspuffer
	Acetonitrile	Azetonitril
	Serum	Humanserum
	Specimen diluent	Probenverdünner
	Verdunningsbuffer	Verdünnungspuffer
	Antiserum	Antiserum
	Immunoabsorbent	Immunadsorbens
	Kalibratorverdunner	Kalibratorverdünnung
	Reconstitutieoplossing	Rekonstitutionslösung
	Polyethyleen glycol	Polyethylenglykol
	Extractieoplossing	Extraktionslösung
	Elutieoplossing	Eluierungslösung
	Bond Elut Silica kolom	Bond Elut Silikakartuschen
	Pre-behandelingsoplossing	Vorbehandlungslösung
	Neutralisatieoplossing	Neutralisierungslösung
	Tracerbuffer	Tracer-Puffer
	Microtiterplaat	Mikrotiterplatte
	HRP Conjuaat	HRP Konjugat
	HRP Conjuaat	HRP Konjugat
	HRP Conjuaat geconcentreerd	HRP Konjugat Konzentrat
	HRP Conjuaat geconcentreerd	HRP Konjugat Konzentrat
	Conjuaat buffer	Konjugatpuffer
	Chromogene TMB geconcentreerd	Chromogenes TMB Konzentrat
	Chromogene Oplossing TMB	Farblösung TMB
	Substraatbuffer	Substratpuffer
	Stopoplossing	Stopplösung
	Incubatieserum	Inkubationsserum
	Buffer	Puffer
	AP Conjuaat	AP Konjugat
	Substraat PNPP	Substrat PNPP
	Geconcentreerd Biotine conjuaat	Biotin-Konjugat-Konzentrat
	Geconcentreerd Avidine-HRP conjuaat	Avidin-HRP-Konzentrat
	Assay buffer	Assaypuffer
	Biotine conjuaat	Biotin-Konjugat
	Specifiek antilichaam	Spezifischer Antikörper
	Streptavidine-HRP concentraat	HRP Streptavidinkonzentrat
	Aspecifieke binding	Unspezifische Bindung
	2de antilichaam	Sekundärer Antikörper
	Verzuringbuffer	Ansäuerungspuffer

	<u>Simboli utilizzati</u>	<u>Símbolos utilizados</u>
	Consultare le istruzioni per l'uso	Consultar las instrucciones de uso
	Limitazioni di temperatura	Limitación de temperatura
	Utilizzare entro	Fecha de caducidad
	Numero di lotto	Código de lote
	Numero di catalogo	Número de catálogo
	Controllo	Control
	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico in vitro
	Fabbricante	Fabricante
	Contenuto sufficiente per <n> saggi	Contenido suficiente para <n> ensayos
	Tampone di lavaggio concentrato	Solución de lavado concentrada
	Calibratore zero	Calibrador cero
	Standard #	Calibrador #
	Controllo #	Control #
	Marcato	Trazador
	Marcato	Trazador
	Marcato concentrato	Trazador concentrada
	Marcato concentrato	Trazador concentrada
	Provette	Tubos
	Tampone incubazione	Tampón de incubación
	Acetonitrile	Acetonitrilo
	Siero	Suero
	Diluente campione	Diluyente de Muestra
	Tampone diluizione	Tampón de dilución
	Antisiero	Antisuero
	Immunoassorbente	Inmunoadsorbente
	Diluente calibratore	Diluyente de calibrador
	Soluzione di ricostituzione	Solución de Reconstitución
	Polietilenglicole	Glicol Polietileno
	Soluzione di estrazione	Solución de extracción
	Soluzione di eluizione	Solución de elución
	Cartucce di silice bond elut	Cartuchos Bond Elut Silica
	Soluzione di pretrattamento	Solución de Pre-tratamiento
	Soluzione di neutralizzazione	Solución de Neutralización
	Tracer Buffer	Tampón de trazador
	Piastra di microtitolazione	Placa de microvaloración
	HRP Coniugato	HRP Conjugado
	HRP Coniugato	HRP Conjugado
	HRP Coniugato concentrato	HRP Conjugado concentrada
	HRP Coniugato concentrato	HRP Conjugado concentrada
	Buffer coniugato	Tampón de Conjugado
	Cromogena TMB concentrato	Cromógena TMB concentrada
	Soluzione cromogena TMB	Solución Cromógena TMB
	Tampone substrato	Tampón de sustrato
	Soluzione di arresto	Solución de Parada
	Incubazione con siero	Suero de Incubación
	Buffer	Tampón
	AP Coniugato	AP Conjugado
	Substrato PNPP	Sustrato PNPP
	Concentrato coniugato con biotina	Concentrado de conjugado de biotina
	Concentrato avidina HRP	Concentrado avidina-HRP
	Soluzione tampone per test	Tampón de ensayo
	Coniugato con biotina	Conjugado de biotina
	Anticorpo Specifico	Anticuerpo específico
	Streptavidina-HRP concentrata	Estreptavidina-HRP Concentrado
	Legame non-specifico	Unión no específica
	2° Anticorpo	Segundo anticuerpo
	Tampone Acidificante	Tampón de Acidificación

	<u>Símbolos utilizados</u>	<u>Använda symboler</u>
	Consulte instruções de utilização	Läs instruktionerna före användning
	Temperatura de conservação	Förvaringstemperatur
	Utilizar antes de	Används av
	Código de lote	Lotnummer
	Número de catálogo	Katalognummer
	Controlo	Kontroll
	Dispositivo médico de diagnóstico in vitro	In vitro diagnostiskt kit
	Fabricante	Tillverkare
	Conteúdo suficiente para <n> testes	Innehållet räcker till <n> prover
	Solução de lavagem concentrada	Tvättlösning, koncentrerad
	Calibrador zero	Nollkalibrerare
	Calibrador #	Kalibrator #
	Controlo #	Kontroll #
	Marcador	Radioisotop, antigen
	Marcador	Radioisotop, antikropp
	Marcador concentrada	Radioisotop, antigen koncentrerad
	Marcador concentrada	Radioisotop, antikropp koncentrerad
	Tubos	Rör
	Tampão de incubação	Inkuberingsbuffert
	Acetonitrilo	Acetonitril
	Soro	Serum
	Diluidor de espécimes	Spädningsbuffert för prover
	Tampão de diluição	Spädningsbuffert
	Anti-soro	Antiserum
	Imunoadsorvente	Immunoadsorberare
	Diluyente do calibrador	Kalibratordiluent
	Solução de Reconstituição	Rekonstitutionslösning
	Polietileno-glicol	Polyetylglykol
	Solução de Extracção	Extraktionslösning
	Solução de Eluição	Elueringslösning
	Cartuchos de sílica Bond Elut	Silikonpatroner för elueringsbindning
	Solução de pré-tratamento	Förbehandlingslösning
	Solução de neutralização	Neutraliseringslösning
	Tampão Marcador	Tracerbuffert
	Placa de micro titulação	Microtiterplatta
	HRP Conjugação	HRP-konjugat
	HRP Conjugação	HRP-konjugat
	HRP Conjugação concentrada	HRP-konjugat-koncentrat
	HRP Conjugação concentrada	HRP-konjugat-koncentrat
	Conjuge o tampão	Konjugatbuffert
	Cromogénica TMB concentrada	Kromogeniskt TMB-koncentrat
	Solução Cromogénica TMB	Kromogenisk TMB-lösning
	Tampão de substrato	Substratbuffert
	Solução de Paragem	Stopplösning
	Soro de incubação	Inkubationsserum
	Tampão	Buffert
	AP Conjugação	AP-konjugat
	Substrato PNPP	Substrat-PNPP
	Concentrado conjugado de biotina	Biotinkonjugat koncentrat
	Concentrado HRP de avidina	Avidin HRP-koncentrat
	Tampão de ensaio	Provbuffert
	Conjugado de biotina	Biotinkonjugat
	Anticorpo específico	-
	Estreptavidina HRP concentrado	-
	Ligações não específicas	-
	Anticorpo secundário	-
	Tampão de acidificação	-

	<u>Επεξήγηση συμβόλων</u>	<u>Anvendte symboler</u>
	Συμβουλευτείτε τις οδηγίες χρήσης	Læs brugsvejledningen
	Θερμοκρασία αποθήκευσης	Opbevaringstemperatur
	Ημερομηνία λήξης	Anvend inden
	Αριθμός παρτίδας	Batchkode
	Αριθμός καταλόγου	Katalognummer
	Πρότυπο ελέγχου	Kontrol
	In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν	Medicinsk udstyr til in vitro-diagnosticering
	Κατασκευαστής	Fabrikant
	Περιεχόμενο επαρκές για «n» εξετάσεις	Indeholder nok til <n> test
	Συμπυκνωμένο διάλυμα έκπλυσης	Koncentreret vaskeopløsning
	Μηδενικός βαθμονομητής	Nul-kalibrator
	Βαθμονομητής #	Kalibrator nr.
	Ορός ελέγχου #	Kontrol nr.
	Ιχνηθέτης	Markør
	Ιχνηθέτης	Markør
	Χρωμογόνος Ιχνηθέτης	Koncentreret markør
	Χρωμογόνος Ιχνηθέτης	Koncentreret markør
	Σωληνάρια	Tuber
	Ρυθμιστικό διάλυμα επώασης	Inkubationsbuffer
	Ακετονιτρίλιο	Acetonitril
	Ορός	Serum
	Διάλυμα αραίωσης δειγμάτων	Prøvediluent
	Ρυθμιστικό διάλυμα αραίωσης	Fortyndingsbuffer
	Αντιορός	Antiserum
	Ανοσοπροσροφητικό	Immonoadsorbent
	Αραιωτικό βαθμονομητών	Kalibratordiluent
	Διάλυμα ανασύστασης	Rekonstitueringsopløsning
	Πολυαιθυλενογλυκόλη	Polyetylenglykol
	Διάλυμα εκχύλισης	Ekstraktionsopløsning
	Διάλυμα έκλουσης	Elueringsopløsning
	Φύσιγγες πυριτίου Bond Elut	Patroner med bindingselueringssilica
	Διάλυμα προεπεξεργασίας	Forbehandlingsopløsning
	Διάλυμα εξουδετέρωσης	Neutraliseringsopløsning
	Ρυθμιστικό διάλυμα	Markørbuffer
	Πλάκα μικροτιτλοδότησης	Mikrotiterplade
	HRP Σύζευγμα	HRP-konjugat
	HRP Σύζευγμα	HRP-konjugat
	Χρωμογόνος HRP Σύζευγμα	HRP-konjugat-koncentreret
	Χρωμογόνος HRP Σύζευγμα	HRP-konjugat-koncentreret
	Ρυθμιστικό διάλυμα συζεύγματος	Konjugatbuffer
	Χρωμογόνος TMB	Kromogen TMB-koncentreret
	Διάλυμα χρωμογόνου TMB	Kromogen TMB-opløsning
	Ρυθμιστικό διάλυμα υποστρώματος	Substratbuffer
	Ανασχετικό αντιδραστήριο	Stopopløsning
	Ορός επώασης	Inkubationsserum
	Ρυθμιστικό διάλυμα	Buffer
	AP Σύζευγμα	AP-konjugat
	PNPP υποστρώματος	Substrat PNPP
	Συμπυκνωμένο αντιδραστήριο συζευγμένο με βιοτίνη	Biotin konjugat koncentrat
	Συμπυκνωμένο διάλυμα αβιδίνης-HRP	Avidin HRP koncentrat
	Ρυθμιστικό διάλυμα προσδιορισμού	Prøvebuffer
	αντιδραστήριο συζευγμένο με βιοτίνη	Biotin konjugat
	Ειδικό Αντίσωμα	-
	Συμπυκνωμένη στρεπταβιδίνη συνεξευγμένη με HRP	-
	μη-ειδική δέσμευση	-
	2ο Αντίσωμα	-
	Ρυθμιστικό Διάλυμα όξινο	-

	<u>Stosowane symbole</u>	<u>Használt szimbólumok</u>
	Przed zastosowaniem zapoznać się z instrukcją	Olvassa el a használati útmutatót
	Temperatura przechowywania	Tárolási hőmérséklet
	Zużyć przed	Lejáratí idő
	Kod serii	Gyártási kód
	Numer katalogowy	Katalógus szám
	Kontrola	Kontrol
	Urządzenie medyczne do diagnostyki in vitro	In vitro diagnosztikai eszköz
	Producent	Gyártó
	Zawartość wystarczająca do <n> testów	Tartalma <n> teszt elvégzésére elegendő
	Roztwór płuczący stężony	Mosó folyadék koncentrátum
	Kalibrator zerowy	Zero kalibrátor
	Kalibrator nr	Kalibrátor #
	Kontrola nr	Kontrol #
	Znacznik izotopowy	Nyomjelző izotóp
	Znacznik izotopowy	Nyomjelző izotóp
	Znacznik izotopowy stężony	Nyomjelző izotóp koncentrátum
	Znacznik izotopowy stężony	Nyomjelző izotóp koncentrátum
	Probówki	Csővek
	Wymagana inkubacja buforu	Inkubáló puffer
	Acetonitryl	Acetonitril
	Surowica	Szérum
	Rozcieńczalnik próbki	Mintahígító
	Bufor do rozcieńczania	Hígító puffer
	Antysurowica	Antiszérum
	Immunoabsorbent	Immunadszorbens
	Rozcieńczalnik kalibratora	Kalibrátor hígító
	Roztwór do rozcieńczania	Mintaelőkészítő oldat
	Glikol poli(oksy)etylenowy	Polietilén glikol
	Roztwór ekstrakcyjny	Extrakciós oldat
	Roztwór elucyjny	Eluáló oldat
	Kolumny krzemionkowe Bond Elut	Bond Elut Silica szilikagél patronok
	Roztwór do przygotowania wstępnego	Előkezelő oldat
	Roztwór neutralizujący	Semlegesítő oldat
	Bufor znacznika	Nyomjelző izotóp hígító puffer
	mikro płytki	Mikrotiter lemez
	Koniugat peroksydazy chrzanowej	HRP konjugátum
	Koniugat peroksydazy chrzanowej	HRP konjugátum
	Koncentrat koniugatu peroksydazy chrzanowej	HRP konjugátum koncentrátum
	Koncentrat koniugatu peroksydazy chrzanowej	HRP konjugátum koncentrátum
	Bufor do koniugacji	Konjugátum puffer
	Koncentrat chromogenu TMB (czterometylobenzodyny)	Kromogén TMB koncentrátum
	Roztwór chromogenu TMB (czterometylobenzodyny)	Kromogén TMB oldat
	Bufor substratu	Szubsztrát puffer
	Roztwór zatrzymujący reakcję	Stop oldat
	Wymagana inkubacja surowicy	Inkubációs szérum
	Bufor	Puffer
	Koniugat AP (fosfatazy alkalicznej)	AP konjugátum
	p-nitrofenylofosforan substratowy	Szubsztrát PNPP
	Koncentrat koniugatu biotyny	Biotin konjugátum koncentrátum
	Koncentrat peroksydazy chrzanowej z awidyną	Avidin HRP koncentrátum
	Bufor do oznaczania	Vizsgálati puffer
	Koniugatu biotyny	Biotin konjugátum
	Przeciwciało swoiste	Specifikus ellenanyag
	Koncentrat streptawidyny HRP	Sztreptawidin HRP koncentrátum
	Wiązanie nieswoiste	Nem-specifikus kötődés
	Drugie przeciwciało	Másodlagos ellenanyag
	Bufor zakwaszający	Savas puffer

	<u>Използвани символи</u>		
	Вижте инструкцията за работа		
	Температура на съхранение		
	Използвайте с		
	Партиден код		
	Каталожен номер		
	Контрол		
	Ин витро диагностично медицинско изделие		
	Производител		
	Съдържание достатъчно за <n> теста		
	Концентриран измиващ разтвор		
	Нулев калибратор		
	Калибратор #		
	Контрол #		
	Трейсър		
	Трейсър		
	Концентриран маркер		
	Концентриран маркер		
	Епруветки		
	Инкубационен буфер		
	Ацетонитрил		
	Серум		
	Разредител за пробите		
	Буфер за разреждане		
	Антисерум		
	Имуноабсорбент		
	Разредител за калибратора		
	Пресъздаващ разтвор		
	Полиетилен гликол		
	Екстрактов разтвор		
	Разтвор за елюиране		
	Силикагелни пълнители		
	Пред-лечебен разтвор		
	Неутрализиращ разтвор		
	Маркерен буфер		
	Микротитърна пластина		
	HRP конюгат / Конюгат на хрянова пероксидаза		
	HRP конюгат / Конюгат на хрянова пероксидаза		
	HRP конюгиран концентрат		
	HRP конюгиран концентрат		
	Буфер за конюгата		
	Хромогенен TMB концентрат		
	Хромогенен TMB разтвор		
	Субстратен буфер		
	Стоп разтвор		
	Инкубационен серум		
	Буфер		
	AP конюгат / конюгат на алкална фосфатаза		
	Субстрат PNPP / пара нитрофенил фосфат		
	Биотин конюгиран концентрат		
	Авидин HRP концентрат		
	Буфер за пробите		
	Биотин конюгат		
	специфично анти тяло		
	стрептавидин HRP концентрат		
	не специфично свързване		
	второ анти тяло		
	киселинизиращ буфер		