

IGFBP-2-ELISA

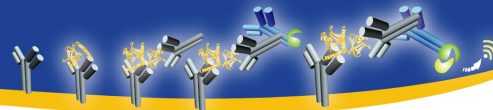
Enzyme Immunoassay for the Quantitative Determination of
Insulin-like Growth Factor Binding Protein-2

40-056-205064

Research Use Only

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TECHNICAL FEATURES

Measures total IGFBP-2 concentration in serum, plasma and in other human body fluids
 Single standards with **2; 10; 20; 40 and 80 ng/ml**
 Total Incubation time only **1.75** hours
 2 Control Sera are provided for quality control purposes according GLP
 Ready-to-use Antibody Conjugate
 No sample extraction needed
 Microtiterplate separately break apart

INTENDED USE

Measurement of human IGFBP-2 in human serum, EDTA-plasma, cerebrospinal fluid, breast milk, amniotic fluid, saliva and in cell culture medium.

CLINICAL IMPLICATIONS

The IGFBP-2 concentration is age-dependent in blood (3).
 Normal values for healthy individuals (1.5 to > 70 years) were evaluated for this assay.
 Supplementary parameter to IGFBP-3 in the diagnosis of growth disorders (IGFBP-2/IGFBP-3 ratio), IGFBP-2 is an inhibitor of growth hormone action (3,4).
 Progression-dependent tumor marker in leukaemia (5), astrocytic CNS tumors (6,7), prostate- (8), suprarenal cortex-(9)-, hepatocellular (10) and other carcinomas.
 Anti-aging parameter: IGFBP-2 as a marker of physiological functionality (20).

INTRODUCTION


Insulin-like growth factors (IGFs) regulate the proliferation, differentiation, apoptose, cell adhesion and metabolism in various tissues and cell types. The IGF-I, which is produced mainly in liver under the influence of growth hormone (GH), regulates as hormone the linear growth of the bones and the process of sexual maturity, while IGF-II is mainly a growth factor of fetal tissue (11-13). The biological actions of IGF over the IGF-Type-I receptor are modulated variably through the IGF binding proteins (IGFBP-1 to-6) (14). IGFBP-2 is, after IGFBP-3, the second most frequent IGFBP in the human blood. IGFs, especially tumor typical pro-IGF-forms and hormones regulate the expression of IGFBP-2, GH effect is thereby inhibiting. At cellular level IGFBP-2 seems to stimulate the proliferation and dissemination of solid tumors via an IGF-independent mechanism (15,16).

PHYSIOLOGICAL MEANING

IGFBP-2 is a unglycosylated polypeptide of 31.3 kDa, which forms binary IGF-complexes and shows no circadian rhythm in the circulation. The serum concentration of IGFBP-2 increases in fasting, after major surgery and after trauma, but the increasing of the concentration is most intensive in malignant diseases. The correlation of the IGFBP-2 level to the degree of progression is a striking feature in various tumor types as is the normalization of the IGFBP-serum levels after remission (5-10). During the GH-therapy, e.g. in short stature and in GH-abuse (doping) the IGFBP-2 level decreases. In Trisomy 18 IGFBP-2 in maternal serum is decreased and IGFBP-1 is increased; therefore the ratio IGFBP-2 /IGFBP-1 is a marker for this chromosome abnormality (17).

MATERIALS

Materials Provided

- 1)  **Microtiter plate**, ready for use: **Microtiter plate** with 96 wells, divided up in 12 strips with 8 wells separately breakable, coated with anti-IGFBP-2 antibody and packed in a laminate bag.
- 2)

CAL	N
-----	---

Calibrators 1-5, lyophilized: contain recombinant IGFBP-2: Calibrator values are between 2 - 80 ng/ml (2, 10, 20, 40, 80 ng/ml) IGFBP-2 and have to be reconstituted with **750 µl Dilution Buffer** each.
- 3)

CONTROL	N
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2 Controls, lyophilized: Contains human serum and has to be reconstituted with **100 µl Dilution Buffer**. The exact concentration of IGFBP-2 is given on the vial label.
- 4)

DIL	BUF
-----	-----

Dilution Buffer, 50 ml, ready for use.
- 5)

Ab	BIOT
SAV	HRP

Antibody-Conjugate, 12 ml, ready for use, contains a mixture of biotinylated anti-IGFBP-2 antibody and HRP (Horseradish Peroxidase)-labelled Streptavidin. Use 100 µl/well in the assay.
- 6)

WASH	SOLN	CONC
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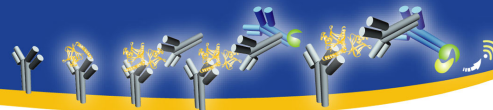
Washing Buffer, 50 ml, 20-fold concentrated: The **Washing Buffer** has to be diluted 1:20 with distilled water or demineralised water before use (e.g. add the complete contents of the flask (50 ml) into a graduated flask and fill up with A.dest. to 1000 ml). Please dilute only according to requirements. The diluted washing buffer is stable for 4 weeks at 2-8°C. It has to be at room temperature for usage !
- 7)

CHROM	TMB
-------	-----

TMB-Substrate Solution 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H₂O₂ Tetramethylbencidine.
- 8)

STOP	SOLN
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Stopping Solution, 0.2 M sulphuric acid, 12 ml, ready for use. *Caution Acid !*
- 9) **Sealing tape** for covering of the microtiter plate, 2 x



Materials 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
- Micropipettes and multichannel pipettes with disposable plastic tips
- Vortex-mixer
- Device to aspirate the calibrators and the samples from the wells (recommended because of the potential danger of infection by human samples)
- Plate washer and plate shaker (≥ 350 rpm) (recommended)
- Calibrated Microplate reader ("ELISA-Reader") with filter for 450/620nm wavelength
- Foil welding device for laminate bags (recommended)
- Timer (120 min. range)
- Reservoirs (disposable)

WARNING AND PRECAUTIONS

The kit should not be used beyond the expiration date on the kit label.

All reagents are for in vitro use only!

In conducting the assay, follow strictly the test protocol. The acquisition, possession and use of the kit are subject to the regulations of the national regulatory authorities.

Reagents with different lot numbers should not be mixed.

Reagents contain as preservative Proclin 950 (containing 0.009025% of 2-methyl-4-isothiazolin-3-one) and Kathon CG (containing 0.001615% of the mixture 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one). They can cause sensitisation by skin contact. (R 43 and S 26-36/37/39-45).

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. The Stop Solution provided is an acid solution. Avoid direct contact. Wear eye, hand, face and clothing protection when using this material.

The handling of potentially infectious material must comply with the following guidelines:

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.

METHODOLOGY

Principle

The GenWay IGFBP-2 40-056-205064 Elisa kit is a so-called Sandwich-Assay using two specific and high affinity antibodies. The IGFBP-2 in the samples binds to the first antibody coated on the microtiter plate. In the following step the second specific anti-IGFBP-2-Antibody binds in turn to the immobilised IGFBP-2. 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 IGFBP-2-level of the samples.

Sample Preparation and Storage

Serum and plasma samples as well as cell culture medium, breast milk, amniotic fluid, cerebrospinal fluid and saliva are applicable.

The blood sample for serum preparation should be gained 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.

Blood samples may be taken at any time of the day.

Storage at RT max. 2 days

Storage at -20°C max. 2 years

Are not allowed to have more than 10 freeze/thaw cycles.

Sample preparation

Samples have to be diluted 1:10-30-fold with Dilution Buffer.

For clinical purposes we recommend a standard dilution of **1:21**.

Suggestion for dilution protocol:

Mix 15 µl serum manually or with the aid of a dilutor with 300 µl Dilution Buffer (1:21). Use 2 x 100 µl of this dilution in the assay or pipette 100 µl buffer in wells and add 5 µl serum.

IGFBP-2 concentrations may be completely different in body fluids of human origin other than serum or cell culture supernatant (s. Table 5).

Technical hints

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

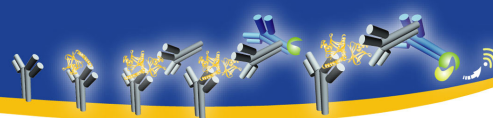
Reagents with different lot numbers cannot be mixed.

Calibrators and Controls

For the reconstitution of the lyophilised components (calibrators 1-5 and Control Sera), 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 calibrator and controls can be stored for 2 months at -20°C . Repeated freeze/thaw cycles have to be avoided.

The Microtiterplate and all reagents are stable until the expiry date if stored in the dark at $2-8^{\circ}\text{C}$ (see label). Store the unused seal stripes of the microtiterplate together with the desiccant at $2-8^{\circ}\text{C}$.



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.

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!

For reconstitution of lyophilized components the kit **Dilution Buffer** should 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 should result), with a Vortex mixer.

The reconstituted standard and controls can be stored for 2 month at –20°C..Avoid repeated freeze-thaw cycles.

Room temperature incubation means: incubation at 20 - 25°C.

ASSAY PROCEDURE

All determinations (Calibrators, Control Sera 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 1-5 and Control Serum** should be pipetted as fast as possible (e.g., 15 Minutes). Antibody-Conjugate and the **Substrate solution** should be added to the plate in the same order and the same time interval as the samples. **Stop Solution** should be added to the plate in the same order as the **Substrate Solution**.

- 1) Add **100 µl** Dilution Buffer to the first wells (blank). Subsequently, add **100µl** of each Calibrator or diluted **Control (1 & 2)** or diluted **Sample** to the following wells.
- 2) Cover the wells with sealing tape and incubate the plate for **1 hour** shaking at room temperature at **≥ 350 rpm**.
- 3) After incubation aspirate the contents of the wells into a disinfectant (risk of infection!) and wash the wells 3 times with **250 µl** of **Washing Buffer** / well respectively. The **Washing Buffer** should incubate at least for **15 seconds**/well.
- 4)Following the last washing step pipette **100µl** of the **Antibody-Conjugate** in each well. and incubate the plate for **30 Minutes** shaking at **room temperature at ≥ 350 rpm**.
- 5) After incubation wash the wells 3 times with **Washing Buffer** as described above.
- 6) Pipette **100 µl** of the **TMB-substrate** solution in each well.
- 7) Incubate the plate for **15 minutes** in the dark at **room temperature**.
- 8) Stop the reaction by adding **100 µl** of **Stopping Solution** to all the wells.
- 9) Measure the absorbance within **30 minutes** at **450 nm** (reference filter: **≥ 590 nm**).

EVALUATION OF RESULTS

Establishing the Calibration Curve:

The calibrators provided contain the following concentrations of IGFBP-2:

Standard	A	B	C	D	E
Ng/ml	2	10	20	40	80

- 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 absorbance of all other values.
- 3) Plot the calibrators concentrations 1-5 on the x-axis versus the mean value of the absorbance of the calibrators on the y-axis. Read the concentration of each control and sample by interpolation on the calibration curve.
- 4) Recommendation: Calculation of calibration curve and sample concentrations should be done by using a computer programme, because the standard curve is in general best described by non-linear regression, a higher-grade polynom or four parametric logistic (4-PL) curve fit are in general suited for the evaluation.
- 5) The IGFBP-2 concentration of the diluted sample or the diluted control sera 1&2 in ng/ml is calculated in this way, the IGFBP-2 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 IGFBP-2 concentration of undiluted sample:

Measured extinction of your sample 0.37
 Measured extinction of the blank 0.06

Your **measurement program** will calculate the IGFBP-2 concentration of the diluted sample automatically by using the difference (0.31) 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 program to calculate the IGFBP-2 concentration in the sample:

$$0.31 = -0.0012048 + 0.039581x + 5.1788 \cdot 10^{-0.005} \cdot x^2 - 1.8929x \cdot 10^{-0.06} \cdot x^3$$

$$7.93 = x$$

if the dilution factor (**1:21**) is taken into account the IGFBP-2 concentration of the undiluted sample is 7.93 x 21= 166.55 ng/ml

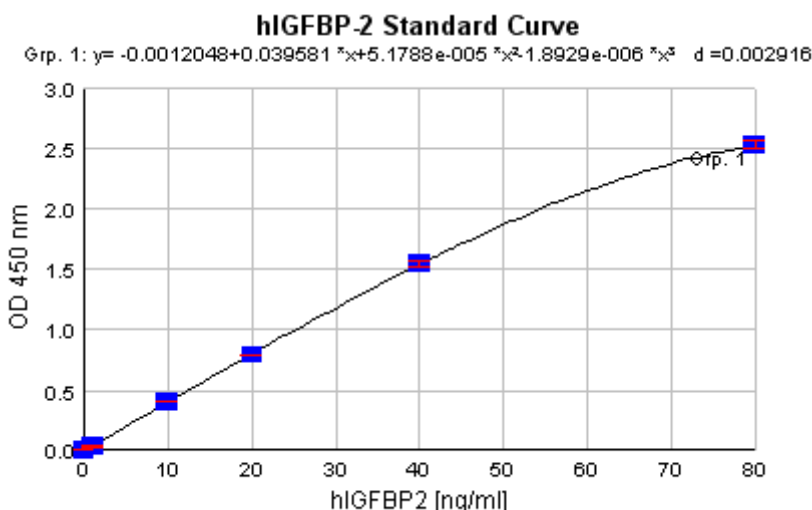
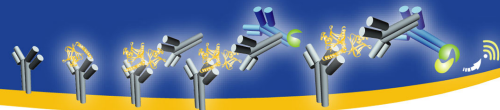


Fig. 1: Exemplary Calibration Curve with a polynomial 3rd degree as curve fit.

PERFORMANCE CHARACTERISTICS

Standards

The Standards of the IGFBP-2 Elisa 40-056-205064 are prepared from **human IGFBP-2** in concentrations of 2, 10, 20, 40 and 80 ng/ml.

Sensitivity

The analytical sensitivity of the assay yields 0.2 ng/ml (2x SD of zero calibrator)

Specificity

This assay is specific for human IGFBP-2, only low degree of cross reactions was found with dog, horse, donkey, cat and goat. No cross-reactivity was with pig, bovine, rabbit, mouse, chicken, rat, guinea pig, sheep.

There is no cross-reactivity with IGFBP-1 nor IGFBP-3

Interference

Interference of bilirubin and triglycerides was tested by adding different amounts of these substances to human serum containing IGFBP-2. For comparison the same amount of buffer without any substance was also added to the serum. Table 1 demonstrates that neither bilirubin nor triglycerides exert any influence on the measurement of IGFBP-2 in human serum.

Table 1: Interference

Bilirubin		Triglycerides	
[µg/ml]	% of control	[mg/ml]	% of control
25	95.07	12.5	100.79
50	92.80	25	101.01
100	93.83	50	103.65
200	88.15	100	101.34

Recovery

Recombinant IGFBP-2 was added in three different concentration to human serum. The IGFBP-2 concentration was measured and the mean relative recovery in comparison to buffer was 108%. Some exemplary data are shown in table 2.

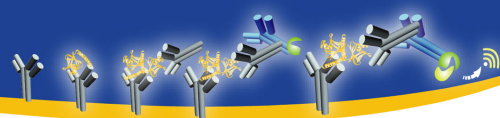
Table 2: Recovery of recombinant human IGFBP-2 in Serum

NIBSC IGFBP-2 [ng/ml]	+1000 ng/ml	+500 ng/ml	+100 ng/ml	Mean [ng/ml]
% Recovery	100,00	112,00	114,00	108,67

Reproducibility and Precision

The inter- and intra assay coefficients of variability are below 10%. Exemplary determinations are shown in table 3 and 4.

Table 3 : Interassay-Variation



Sample 1 (ng/ml)	137	159	152
Sample 2 (ng/ml)	672	697	688
Sample 3 (ng/ml)	928	929	956

Table 4: Intra-Assay-Variation

Sample 1 ng/ml	322	375	298	305	318	311	320	325	302	301	305	317
Sample 2 ng/ml	612	609	616	648	594	597	620	613	617	611	636	698

Table 5: Linearity of the sample dilution:

Dilution	Serum 1 (ng/ml)	Serum 2 (ng/ml)	Cerebrospinal fluid (ng/ml)	Amniotic fluid (ng/ml)
1:10	938	582	426	Not determined
1:20	1061	673	428	460
1:40	1055	719	379	483
1:80	1004	691	318	431
1:160	952	668	426	415

EXPECTED VALUES

The IGFBP-2 concentration in serum is depended on age (Table 8) and on Body Mass Index (BMI; Table 7). For data collection of these reference values IGFBP-2 levels were determined in serum of over 400 normal children and adults (see table 8 figure 2); (3).

Please see the expected values of IGFBP-2 levels in other human body fluids than serum and in cell culture medium in the table 6.

Table 6: Expected values of IGFBP-2 in body fluids of human origin and in cell culture supernatants:

Sample	Expected Value (ng/ml)
serum	[100 - 1000]
liquor	[100 - 300]
amniotic fluid	[200 - 10000]
seminal plasma	[5000 - 15000]
breast milk	[1500-3000]
cell culture supernatants	[5 - 300]

Table 7: BMI dependent reference values, adults between 20 and 80 years

BMI [kg/m ²]	N	IGFBP-2 [ng/ml]		percentiles		
		mean	SD	5th	50th	95th
15	12	612	110	431	612	793
17,5	14	568	126	361	568	775
20	76	509	144	271	509	746
22,5	124	449	162	182	449	716
25	101	398	165	127	398	670
27,5	52	348	147	106	348	590
30	25	315	118	120	315	510
32,5	15	282	90	135	282	430
35	4	251	80	119	251	383
37,5	4	220	71	104	220	336

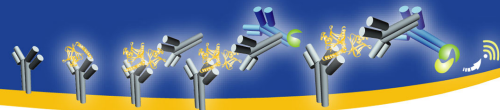
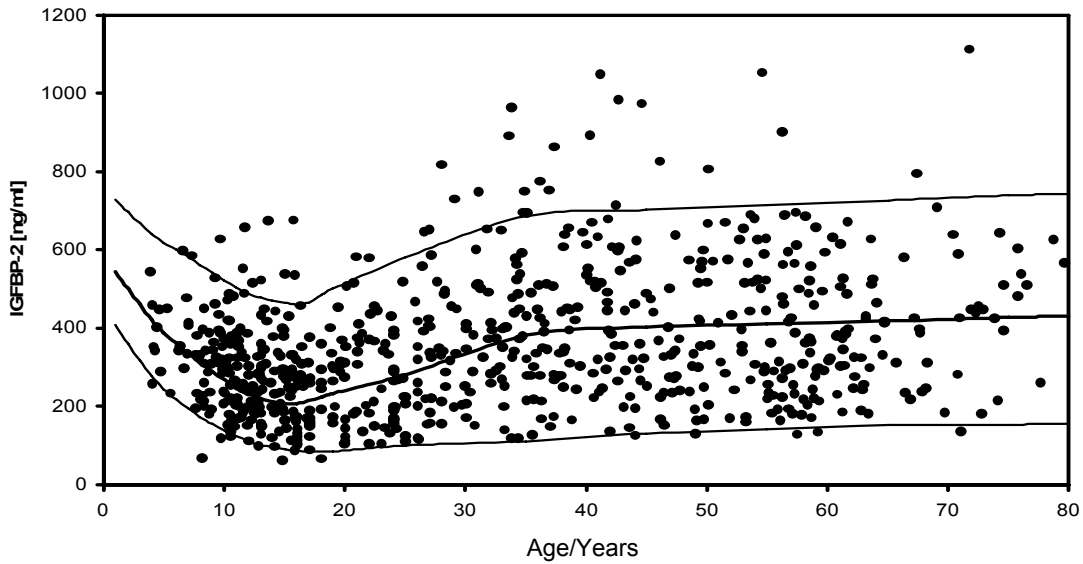
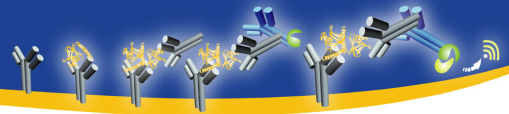


Table 8: IGFBP-2 serum levels (in ng/ml) of > 400 healthy individuals. The normal range is given by the 5., 50. and 95. percentile for age classes.

Age-dependent normal range of serum IGFBP-2

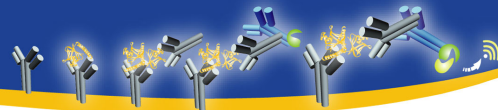
age (years)	5. percentile (ng/ml)	50. percentile (ng/ml)	95. percentile (ng/ml)
1	408	545	728
2	359	500	696
3	317	460	668
4	277	421	640
5	243	388	617
6	217	361	602
7	194	336	583
8	173	312	562
9	154	289	542
10	138	268	522
11	123	249	503
12	111	232	486
13	101	219	477
14	94	212	470
15	89	207	465
16	86	207	460
17	84	214	466
18	84	223	483
19	84	232	500
25	99	280	580
35	110	381	686
45	130	403	702
55	140	410	715
65	151	418	727
75	153	427	740
80	156	430	744



Assembled by Dr. R. Schweizer, Tübingen, Germany

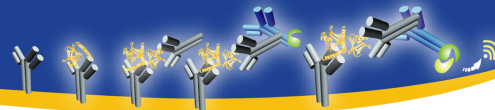
LIMITATION

Deviation from the reference range can be expected especially in hypothyroidism, after major surgery, in polytrauma, in Diabetes mellitus (due to insulin therapy), in fasting and in malignant diseases.



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Reagent:	Reconstitution:	dilution:
Calibrators 1-5	in 750 µl Dilution Buffer	
Control Serum 4 (1 & 2)°	in 100 µl Dilution Buffer	1:21 with Dilution Buffer
Washing Buffer		1:20 with Distilled water (e.g., add the complete contents of the flask (50 ml) into a graduated flask and fill with Distilled water to 1000 ml).
Sample Dilution: Serum samples should be diluted prior to measurement 1:10-30-fold with Dilution Buffer depending on the expected values. In general a dilution of 1:21 is appropriate. Use 2 x 100 µl of this dilution 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 (20 ng/ml)	D1/2
100 µl	Calibrator 4 (40 ng/ml)	E1/2
100 µl	Calibrator 5 (80 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-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
Measure the absorbance within 30 min at 450 nm with ≥ 590 nm as reference wavelength.		

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