Mouse/Rat Growth Hormone ELISA

For the quantitative determination of Growth Hormone in mouse and rat serum or plasma.

For Research Use Only. Not For Use in Diagnostic Procedures.

Catalog Number: 22-GHOMS-E01

Size: 96 wells

Version: 19.12.22 /Rev 003 - ALPCO 1.1

1. Intended use

The Mouse/Rat Growth Hormone ELISA is intended to be used for the quantitative determination of Growth Hormone in mouse and rat serum and plasma samples. For Research Use Only. Not for Use in Diagnostic Procedures.

2. Assay Principle

The Mouse/Rat Growth Hormone ELISA is a sandwich immunoassay. It utilizes two different specific high affinity polyclonal antibodies for this protein. The growth hormone in the sample binds to the immobilized antibody. In the following step, the biotinylated antibody in turn binds to growth hormone. After washing, a streptavidin-peroxidase-enzyme conjugate is added, which will specifically bind to the biotin of the antibody. Subsequently, the peroxidase catalyzes an enzymatic reaction upon the addition of substrate resulting in a blue color. The intensity of the blue color depends on the growth hormone content of the sample. The reaction is stopped by the addition of stop solution and color intensity is quantified by measuring the absorption.

3. Warnings and Precautions

For research use only. Not for Use in Diagnostic Procedures.

The Mouse/Rat Growth Hormone ELISA kit is only suitable for laboratory use and is not for internal use in humans and animals. Strictly follow the test protocol. Use the valid version of the package insert provided with the kit. Be sure everything has been understood. ALPCO will not be held responsible for any loss of damage (except as required by statue) caused because of noncompliance with the instructions provided.

Do not use obviously damaged or microbially contaminated or spilled material.

Caution: This kit contains material of human and/or animal origin. Therefore, all components and samples should be treated as potentially infectious.

Appropriate precautions and good laboratory practices must be used in the storage, handling, and disposal of the kit reagents. The disposal of the kit components must be made according to local and national regulations.

Animal serum: mouse/rat in the following components: KS1, KS2

Reagents AK, EK, VP, WP

Contain 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (< 0.015%) as preservative

H317	May cause an allergic skin reaction
P280	Wear protective gloves/protective clothing/eye protection/face protection
P272	Contaminated work clothing should not be allowed out of workplace
P261	Avoid breathing dust/fumes/gas/mist/vapors/spray
P333+P313	If skin irritation or rash occurs, seek medical advice/attention
P302+P352	If ON SKIN, wash with plenty of soap and water
P501	Dispose of contents/container in accordance with local/regional/national/ international regulations

Substrate Solution (s)

TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine (<0.05%)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

P261 Avoid breathing dust/fume/gas/mist/vapors/spray.
P305+P351+P338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses, if present and easy to do; continue rinsing.

Stop Solution (SL)
The Stop Solution contains 0.2 M Sulfuric acid (H_2SO_4)

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+P330+P331 IF SWALLOWED: rinse mouth. DO NOT induce vomiting.
P305+P351+P338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses, if present and easy to do; continue rinsing.
P309+P310 If exposed and if you feel unwell, immediately call a POISON CENTER or doctor/physician.

3.1 General first aid procedures

Skin contact: Wash affected area immediately and thoroughly with plenty of water for at least 15 minutes. Remove contaminated clothes and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. To assure effective rinsing, spread the eyelids.

Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water and seek medical advice immediately.

4. Materials

4.1 Materials Provided
The reagents listed below are sufficient for 96 wells including the standard curve.

MTP	**Microtiter plate**, ready for use, coated with goat-anti-mouse/rat growth hormone antibodies, wells are separately breakable.	8 x 12 wells
A-G	**Standards, lyophilized, recombinant rat growth hormone** Concentration is given on the vial labels and quality certificate.	7 x 1 mL
VP	**Dilution Buffer VP**, ready for use. **Please shake before use.**	1 x 50 mL
KS1	**Control Serum 1, lyophilized, rat serum** Concentration is given on the quality certificate.	1 x 150 µL
KS2	**Control Serum 2, lyophilized, rat serum** Concentration is given on the quality certificate.	1 x 150 µL
AK	**Antibody Conjugate,** ready for use, guinea pig anti-mouse/rat growth hormone antibody, biotinylated.	1 x 12 mL
EK	**Enzyme Conjugate,** contains HRP (horseradish peroxidase)-labeled streptavidin.	1 x 12 mL
WP	**Wash Buffer WP, 20X concentrated** solution.	1 x 50 mL

S	**Substrate S,** ready for use, HRP substrate, stabilized Tetramethylbenzidine.	1 x 12 mL
SL	**Stop Solution,** ready for use, 0.2 M sulfuric acid, Caution acid!	1 x 12 mL
-	Sealing tape for covering the microtiter plate	3

4.2 Materials required, but not provided

- Precision pipettes and multichannel pipettes with disposable plastic tips
- Graduated cylinder for diluting Wash Buffer (WP)
- Distilled or deionized water for dilution of the Wash Buffer (WP)
- Vortex-mixer
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)
- Microplate reader ("ELISA-Reader") with filter for 450 nm and ≥ 590 nm
- Polyethylene (PE)/Polypropylene (PP) tubes for dilution of samples
- Timer

5. Samples

5.1 Sample Type

Mouse and rat serum and plasma samples are suitable for this assay.

EDTA-plasma samples of rats were found to be increased by plus 100% in a comparative study, relating to rat serum GH-values (Lit. 1).

5.2 Sample Collection

Hemolyzed samples must be avoided.

5.3 Requested sample volume: 20 µL per single determination.

5.4 Sample Stability

- Sample transport is recommended chilled on cooling elements (blue ice) or frozen on dry ice
- Firmly close sample vials
- Storage at -20˚C: 2 years
- Freeze-thaw cycles: max. 10

It is recommended to store samples chilled as soon as possible. For longer storage, the sample must be kept frozen at -20˚C.

5.5 Sample Dilution

For commercial pooled rodent sera, a 1:5 dilution was found to be suitable. An extraction step is not required.

- Dilution: with **Dilution Buffer VP**:
    - For duplicate determinations, add 50 µL sample to 200 µL **Dilution Buffer VP**

- Mix well and add 100 µL of the diluted sample per well in the assay within 1 hour of dilution.
- Where required, depending on the expected growth hormone values, the dilution with **Dilution Buffer VP** may be higher or lower.

Depending upon the strain of the animals or the experimental conditions used, the endogenous content of growth hormone can vary greatly. It is recommended to test in advance and optimize sample dilutions under the respective conditions.

6. Technical Notes

Storage Conditions

- Store the kit at 2-8˚C after receipt until its expiry date.

Storage Life

- The shelf life of the components after initial opening is 4 weeks.
- Store the unused microtiter strips together with the desiccant at 2-8˚C in the airtight clip lock bag and use in the frame provided.
- The 1X working Wash Buffer WP is stable for 4 weeks at 2-8˚C.
- The reconstituted components, standards A-G and Control Sera KS1 and KS2, must be stored at -20˚C (max. 4 weeks). For use, thaw quickly, but avoid temperature increases above room temperature and avoid excessive vortexing. Repeated freeze-thaw cycles should be avoided. Up to three cycles were found to have no influence.

Preparation of Reagents

- Bring all reagents to room temperature (20-25˚C) before use.
- Precipitates present in the buffers must be dissolved prior to use by mixing and/or warming of the buffers.
- Reagents with different lot numbers cannot be mixed.

Reconstitution

- The Standards A-G and Control KS1 and KS2 are reconstituted with Dilution Buffer VP.
- It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly, but gently (avoid foaming) with a vortex mixer.
- **Attention**: Standards should be thawed only once; where required please store aliquoted in adequate volumes.

Dilution

- After reconstitution, dilute the Control Sera KS1 and KS2 with Dilution Buffer VP in the same ratio (e.g. 1:5) as the sample.
- The required volume of 1X working Wash Buffer WP is prepared by 1:20 dilution of the provided 20x concentrate with distilled water.

Incubation

- Incubation at room temperature means: incubation at 20-25˚C.
- Substrate Solution S (stabilized TMB) is photosensitive; store and incubate in the dark.

Assay Procedure

- When performing the assay, the Blank, Standards A-G, Control Serum KS, and the samples should be pipetted as fast as possible (e.g. <15 minutes).
- To avoid variation due to differences in incubation times, Antibody Conjugate AK, Enzyme Conjugate EK, as well and the succeeding Substrate Solution S should be added to the plate in the same order and in the same time interval as the samples. The Stop Solution should be added to plate in the same order as the Substrate Solution S.
- All determinations (Blank, Standards A-G, Control Sera KS1 and KS2, and samples) should be assayed in duplicate.
- For optimal results, accurate pipetting and adherence to the protocol are recommended.

Shaking

- The incubation steps should be performed using a microtiter plate shaker set to 350 rpm. Depending on the design of the shaker, the shaking frequency should be adjusted. Insufficient shaking may lead to inadequate mixing of the solutions and thereby to low optical densities, high variations, and/or false values; excessive shaking may result in high optical densities and/or false values. Substrate S should be incubated without shaking.

Washing

- Proper washing is important for the reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false result calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.
- All washing must be performed with the provided Wash Buffer WP diluted to usage concentration. Washing volume per washing cycle and well must be at least 300 µL. Danger associated with handling of infectious material must be taken into account.
- When using an automatic microtiter plate washer, the respective instructions for use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, remove the remaining fluid and then invert the plate and repeatedly tap it dry on lint-free absorbent tissue.
- Manual washing is an adequate alternative option. Wash Buffer may be dispensed via a multistep device, a multichannel pipette, or a squirt bottle. The fluid may be removed by shaking out the microtiter plate over a basin. If aspirating devices are used, care must be taken that the inside well surface is not scratched. After every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on lint-free absorbent tissue.

7. Assay Procedure

7.1 Preparation of Reagents

Standards A-G

Reconstitute Standards A-G in 1mL of Dilution Buffer VP. Let sit for 15 minutes then then mix thoroughly but gently (without foaming) with a vortex mixer. Standards should be frozen and thawed only once. Aliquot before freezing if required.

Control Serum (KS1 and KS2)
Reconstitute Control Serum (KS1 and KS2) in 150 µL Dilution Buffer VP for a 1:5 Dilution. Mix immediately and incubate max of 60 minutes. Use 100 µL for each well in the assay.

Wash Buffer (WP)
Dilute 1:20 with distilled water.

7.2 Assay Procedure

1. Bring all reagents to room temperature (20-25˚C) before starting the assay.

2. Add 100 µL Dilution Buffer VP into the first two wells (these wells serve as Blanks). Subsequently add 100 µL Standard or 100 µL of diluted Control Sera or diluted samples into wells designated for Standards (A-G), Controls (KS1 and KS2) or samples.

3. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (20-25˚C), shaking at 350 rpm.

4. After incubation, aspirate the contents of the wells and wash the wells 5 times with 300 µL 1 X working Wash Buffer WP per well.

5. Following the last washing step, pipette 100 µL of the Antibody Conjugate AK in each well.

6. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (20-25˚C), shaking at 350 rpm.

7. After incubation aspirate the contents of the well and wash the wells 5 times with 300 µL 1X working Wash Buffer as described in step 4.

8. Following the last washing step pipette 100 µL of the Enzyme Conjugate EK in each well.

9. Cover the wells with sealing tape and incubate the plate for 30 mins at room temperature (20-25˚C), shaking at 350 rpm.

10. After incubation aspirate the contents of the wells and wash the wells 5 times with 300 µL 1X working Wash Buffer as described in step 4.

11. Pipette 100 µL of the TMB Substrate Solution in each well.

12. Incubate the plate for 30 minutes in the dark at room temperature (20 – 25°C).

13. Stop the reaction by adding 100 µL of Stop Solution.

14. Measure the color reaction within 30 minutes at 450 nm (reference filter ≥ 590 nm).

8. Calculation of Results

8.1 Establishing the Standard Curve

For the evaluation of the assay, the absorbance values of the blank should be below 0.30, and those of standard G should exceed 1.0.

Samples which yield higher absorbance values than Standard G are beyond the standard curve, and for reliable determinations these samples should be tested again with a higher dilution.

Standards are provided in the following concentrations:

Standards	**A**	**B**	**C**	**D**	**E**	**F**	**G**
ng/mL	0.15	0.45	0.90	1.8	3.6	6.0	9.0

1. Calculate the **mean absorbance** value for the Blank from the duplicate determinations (well A1/A2).

2. Subtract the mean absorbance of the Blank from the mean absorbance 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.

4. Recommendation: Calculation of the standard curve should be done by using a computer program. **A higher-grade polynomial,** or **four-parametric logistic (4-PL) curve fit** or **non-linear regression** are usually suitable for the evaluation (as might be spline or point-to-point alignment in individual cases).

5. The mouse/rat growth hormone (m/rGH) concentration of the **diluted** sample or the **diluted** control sera KS1&2 in ng/mL can be calculated in this way: the m/rGH concentration of the **undiluted** sample and of KS1 & KS2 is calculated by **multiplication** with the respective dilution factor.

8.2 Example of a Typical Standard Curve

Standards	**Blank**	**A**	**B**	**C**	**D**	**E**	**F**	**G**
ng/mL	0	0.15	0.45	0.90	1.8	3.6	6.0	9.0
$OD_{(450\text{-}620\ nm)}$	0.1356	0.182	0.272	0.4	0.634	1.121	1.732	2.408

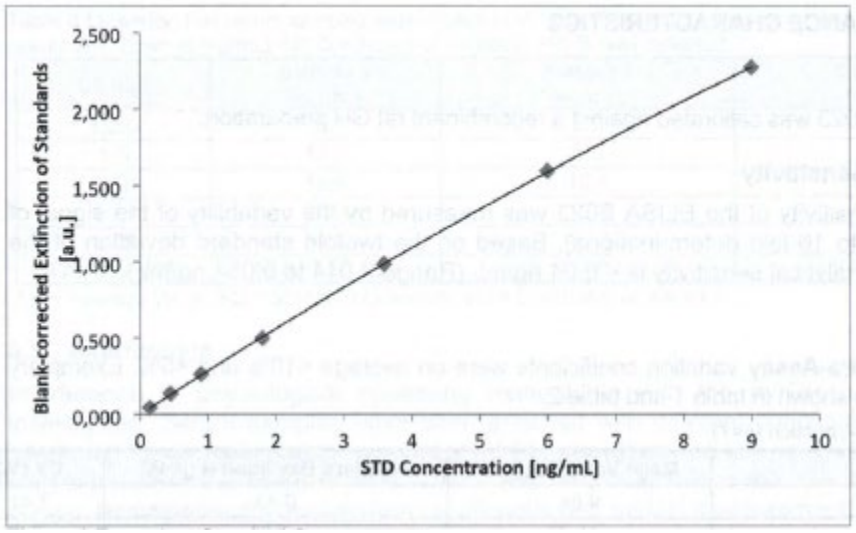

Figure 1 Example Standard Curve

The example standard curve shown in Figure 1 cannot be used for calculation of the test results. A standard curve must be established for each test conducted.

8.3 Example calculation of the mouse/rat growth hormone concentration o fa diluted sample:

Sample dilution: 1:5
OD 450 nm
Measured extinction (mean value) of the sample 1.5
Measured extinction of the blank (mean value) 0.1356

The measurement program will calculate the mouse/rat growth hormone concentration of the sample automatically by using the difference of sample and blank for the calculation. Determine the most suitable curve fit (here: polynomial 2nd degree).

In this example, the following equation is solved by the program to calculate the mouse/rat growth hormone concentration in the sample:

$$y = -0.0041x^2 + 0.2896x$$
$$x = 5.09$$

Multiplication by the dilution factor (5) gives the growth hormone concentration of the sample as 25.45 ng/mL.

9. Performance Characteristics

9.1 Calibration

The Mouse/Rat Growth Hormone ELISA was calibrated against a recombinant rat growth hormone preparation.

9.2 Analytical Sensitivity

The analytical sensitivity of the ELISA was measured by the variability of the signal of the blank (by 15 to 16-fold determinations). Based on the two-fold standard deviation of the blank the mean analytical sensitivity is <0.04 ng/mL (Range 0.014 to 0.054 ng/mL).

9.3 Precision

The inter- and intra-assay variation coefficients were on average <10% and <5%. Example determinations are shown in Table 1 and Table 2:

Table 1: Inter-Assay-Variation (n=7)

	Mean Value(ng/mL)	Standard Deviation (ng/mL)	CV (%)
Sample 1	9.84	0.73	7.41
Sample 2	15.77	0.86	5.48

Table 2: Intra-Assay-Variation (n=12)

	Mean Value(ng/mL)	Standard Deviation (ng/mL)	CV (%)
Sample 1	10.03	0.32	3.22
Sample 2	3.74	0.17	4.55
Sample 3	16.16	0.33	2.01

9.4 Linearity

Linearity of sample dilution was tested by serial dilution of 3 rat sera. No diluted sample showed a relative standard deviation of >15% in comparison to the respective mean rat growth hormone concentration. Linearity of sample dilution is shown by linear regression in the dilution range of 1:2.5 to 1:30. It is recommended to use a dilution of 1:5. Alternatively dilutions from 1:2.5 up to 1:30 (in case of higher growth hormone levels) would be suitable.

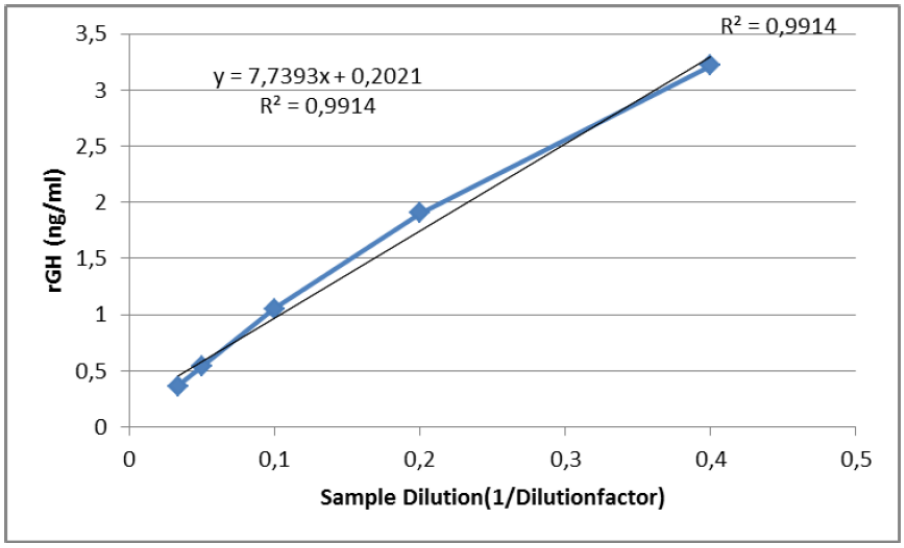

Figure 2 Example regression curve of the dilution (1:2.5 to 1:30) of the rat sample.

Table 3: Linearity. Rat serum samples were diluted in Dilution Buffer VP and rat growth hormone content was calculated. Measurements are shown in ng/mL. No coefficient of variation >15% was detected.

Dilution	Sample 1 (ng/mL)	Sample 2 (ng/mL)	Sample 3 (ng/mL)
1:2.5	15.1	8.0	3.968
1:5	17.7	9.5	4.62
1:10	19.6	10.5	5.329
1:20	20.8	10.8	5.508
1:30	21.3	10.8	
AV / SD / %CV	18.9/ 2.5 / 13.2	9.93 / 1.189 / 11.95	4.9 / 0.7 / 14.4

AV = average value, SD = standard deviation, CV= coefficient of variation

9.5 Interference

Interference of hemoglobin with the mouse/rat growth hormone measurement was investigated. Serum samples were enriched with different concentrations of hemoglobin and compared to the same sample without any added hemoglobin. Table 4 shows the relative results of the experiment. Hemoglobin did not interfere significantly with mouse/rat growth hormone measurement.

Table 4: Recovery [%] in comparison to the native serum.

	Hemoglobin (5 mg/mL)
Sample 1	89
Sample 2	94
Sample 3	114

9.6 Species Cross-Reactivity

Serum of different species were used to dilute samples in this assay system. No cross-reactivity was detected for: rabbit, guinea pig, dog, cat, chicken, sheep, goat, pig, donkey, horse, and bovine.

No cross-reactivity was measured with recombinant human eukaryotic expressed growth hormone at 1 µg/mL.

10. Assay Summary

Mouse/Rat Growth Hormone ELISA	96 Wells
RUO	For Research Use Only
Principle of the test	Enzyme-linked Immunoassay
Incubation Time	3 hours
Antibodies	Specific guinea pig and goat anti-mouse/rat growth hormone antibodies
Buffer	Ready for use, 20x concentrate
Standard	7 single standards (0.15 - 9.0 ng/mL), recombinant rat growth hormone
Assay Range	0.04 – 45 ng/mL
Control	2 Control sera, lyophilized

Sample	Mouse and Rat Serum and Plasma
Sample Dilution	Dependent on available sample volume: preferentially recommended 1:5; acceptable from 1:2.5 up to 1:30
Required Sample Volume for 1:5 Dilution	20 µL net for a single determination
Analytical Sensitivity	<0.04 ng/mL
Intra- and Inter-assay Variance	<5 % / <10%

11. Short Assay Procedure

Preparation of reagents:		**Reconstitution:**	**Dilution:**
A-G	Standards	in 1 mL Dilution Buffer VP	-
KS1	Control Serum 1	in 150 µL Dilution Buffer VP	1:5 with Dilution Buffer VP
KS2	Control Serum 2	in 150 µL Dilution Buffer VP	1:5 with Dilution Buffer VP
WP	Wash Buffer	-	1:20 with distilled water
Sample and Control Sera KS1 and KS2: dilute 1:5 with Dilution Buffer VP, mix immediately, incubate max. 60 min. Use 100 µL for each well in the assay.			
Before assay procedure bring all reagents to room temperature 20-25°C.			
Assay Procedure in Double Determination:			
Pipette	**Reagents**		**Position**
100 µL	Dilution Buffer VP (Blank)		A1/A2
100 µL	Standard A (0.15 ng/mL)		B1/B2
100 µL	Standard B (0.45 ng/mL)		C1/C2
100 µL	Standard C (0.90 ng/mL)		D1/D2
100 µL	Standard D (1.8 ng/mL)		E1/E2
100 µL	Standard E (3.6 ng/mL)		F1/F2
100 µL	Standard F (6.0 ng/mL)		G1/G2
100 µL	Standard G (9.0 ng/mL)		H1/H2
100 µL	Control Serum KS1 (1:5 diluted)		A3/A4
100 µL	Control Serum KS2 (1:5 diluted)		B3/A4
100 µL	Sample (1:5 diluted)		in the rest of the wells according to the requirements
Cover the wells with the sealing tape.			
Sample Incubation: 1 h at 20-25°C, 350 rpm			
5x 300 µL	Aspirate the contents of the wells and wash 5x with 300 µL each 1X working Washing Buffer WP/ well.		In each well
100 µL	Antibody Conjugate AK		In each well
Cover the wells with the sealing tape.			
Incubation: 1 h at 20-25°C, 350 rpm			
5x 300 µL	Aspirate the contents of the wells and wash 5x with 300 µL each 1X working Washing Buffer WP/ well.		In each well

100 µL	Enzyme Conjugate EK	In each well
Cover the wells with the sealing tape.		
Incubation: 30 min at 20-25°C, 350 rpm		
5x 300 µL	Aspirate the contents of the wells and wash 5x with 300 µL each 1X working Washing Buffer WP/ well.	In each well
100 µL	Substrate Solution S	In each well
Cover the wells with the sealing tape.		
Substrate S Incubation: 30 min in the Dark at 20-25°C		
100 µL	Stop Solution SL	In each well
Measure the absorbance within 30 min at 450 nm with ≥ 590 nm as reference wavelength.		

12. References

1. Popp et al (2012). Analysis of different blood sample pre-treatment conditions on hormone concentrations in rats. Quelle/Source: Abstract-CD 55. Symposium der Deutschen Gesellschaft fur Endokrinologie 2012; ISSN 1862-1503.