

Human Relaxin ELISA Kit



Instruction Manual

PromoKine

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Intended Use

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) is intended for the quantitative determination of Relaxin in serum, plasma, urine, cell culture supernatant and tissue samples. **It is for *in vitro* diagnostic use only.**

Introduction

Relaxin is a peptide hormone with a molecular weight of 6500 Da that belongs to the insulin family. Its main function is the relaxation of smooth musculature. Because of the increased **relaxin** levels during ovulation and pregnancy most of the knowledge about its physiological properties is gained in the field of gynecology and reproductive sciences. Recently, novel sites of **relaxin** action have been recognized. In particular, it has been shown that **relaxin**: (i) promotes dilation of blood vessels in several organs and tissues, including the uterus, the mammary gland, the lung and the heart; (ii) has a chronotropic action on the heart; (iii) inhibits the stimulation of endothelin-1, the most potent vasoconstrictor in heart failure. (iv) inhibits the release of histamine by mast cells, thus being able to counteract experimental allergic asthma; (v) depresses aggregation of platelets and their release by megakaryocytes; (vi) influences the secretion of hormones by the pituitary gland; and (vii) contributes to the regulation of fluid balance.

Specific G protein-coupled receptors for **relaxin**, LGR7 and LGR8, have been found in the brain (interaction with ADH-secretion), uterus and heart (effect on the heart frequency). Dschietzig et al. (2004) report that **relaxin** acts as a glucocorticoid-receptor-agonist. Recent publications describe a relationship between **relaxin** and oxidative stress. Bani et al. (1997) and Nistri (2003) demonstrate, that **relaxin** added to reperfusion solutions protects myocardial tissue of ischemic rat hearts against oxidative damage. Moreover, the production of malondialdehyde (degradation product during lipid oxidation) and myeloperoxidase (marker for the activity of granulocytes) has been significantly reduced. As a result, reduced damage of the myocardial tissue during ischemia/reperfusion, and as a consequence, reduced death rates have been observed. Finally, Hocher et al. (2004) found **relaxin** as an independent risk factor predicting death in a survey of

245 male patients with end-stage renal disease (ESRD) on chronic hemodialysis.

Indications

- Determination of the protection efficiency during reperfusion/ ischemia
- Regulation of blood pressure and heart frequency, microcirculation
- Studies of angiogenesis
- Studies of immunomodulation
- Examinations in the area of reproduction medicine
- Predicting factor for survival of ESRD-patients

Material Supplied

Content	Kit Components	Quantity
PLATE	One holder with precoated strips	12 x 8
WASHBUF	ELISA wash buffer concentrate 10x	100 ml
SAMPLEBUF	Sample dilution buffer, ready-to-use	50 ml
AB	Detection antibody, rabbit anti-Relaxin biotin labeled, concentrate	1 vial
STD	Standard concentrate, lyophilized (for range see specification or label)	1 vial
CTRL1 CTRL2	Controls, lyophilized (for range see specification)	2 x 1 vial
CONJ	Conjugate, streptavidin peroxidase labeled antibody, concentrate	1 vial
SUB	TMB substrate (Tetramethylbenzidine), ready-to-use	15 ml
STOP	ELISA stop solution, ready-to-use	15 ml

Material Required but not Supplied

- Bidistilled water (aqua bidest.)
- Precision pipettors and disposable tips to deliver 10-1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm (reference wave length 620 or 690 nm)

Preparation and Storage of Reagents

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before
- The **WASHBUF** (wash buffer concentrate) should be diluted with aqua bidest. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C using a water bath before dilution. The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.
- The **AB** (biotinylated antibody) must be diluted **1:1000** in **wash buffer** (10 µl AB + 10 ml wash buffer). The AB is stable at **2-8 °C** until the expiry date stated on the label. **Diluted antibody solution is not stable and can not be stored.**
- The CONJ (conjugate, streptavidin peroxidase-labeled) must be diluted **1:1000** in **wash buffer** (10 µl CONJ + 10 ml 1x WASHBUF). The undiluted conjugate is stable at **2-8 °C** until the expiry date stated on the label. **Diluted conjugate is not stable and can not be stored.**
- The lyophilized **STD** (standard) is stable at 2-8°C until the expiry date stated on the label. The lyophilized **STD** must be reconstituted with **1 ml** aqua dest. Allow to dissolve for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. The reconstituted standard is stable at 2 - 8 °C for four weeks. Frozen aliquots can be stored at -20°C up to 1 year.
- The STD (standard concentrate, 1000 pg/ml) must be diluted **1:4** with SAMPLEBUF (sample dilution buffer):

200 µl STD-concentrate + 600 µl SAMPLEBUF.

The obtained solution has a concentration of 250 pg/ml (S1, Standard 1).

The solutions for the standard curve are prepared from S1 in **1:3** dilution steps by adding SAMPLEBUF (sample dilution buffer) as follows:

S1 = 250 pg/ml

S2 = 150 µl **S1** + 300 µl SAMPLEBUF = 83 pg/ml

S3 = 150 µl **S2** + 300 µl SAMPLEBUF = 28 pg/ml

S4 = 150 µl **S3** + 300 µl SAMPLEBUF = 9.3 pg/ml

S5 = 150 µl **S4** + 300 µl SAMPLEBUF = 3.1 pg/ml

SAMPLEBUF (sample dilution buffer) is used as standard (0 pg/ml).

- The lyophilized **CTRL1, CTRL2** (controls 1 and 2) are stable at 2-8°C until the expiry date stated on the label. The lyophilized **CTRL1, CTRL2** must be reconstituted with **1 ml** aqua dest. Allow to dissolve for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. The reconstituted controls are stable at 2 - 8 °C for four weeks. Frozen aliquots can be stored at -20°C up to 1 year.
- All other test reagents are ready to use. The test reagents are stable until the expiry date (see label of test package) when stored at **2-8°C**.

Sample Preparation

Serum, plasma, seminal plasma, tissue extract and cell culture supernatant are suited for this assay. The samples should be stored at -20°C until use.

Sample dilution

Serum/Plasma

Serum and plasma samples must be diluted at least **1:3** with SAMPLEBUF (sample dilution buffer).

Serum and plasma samples could contain rheumatoid factor and heterophilic antibodies, which can cause false positive results in sandwich immunoassays. To reduce the potential interference from rheumatoid factor and heterophylic antibodies, the samples can be cleared by treating twice with 5% (v/v) Anti-Interference Reagent (available on request) as follows:

- 10 µl anti interference reagent + 200 µl sample
- shake for 1 hour at 4 °C
- centrifuge and collect the supernatant (pre-cleared/pre-treated sample).

Urine

Urine samples should be diluted at **least 1:4** with SAMPLEBUF (sample dilution buffer).

Seminal plasma

Seminal plasma has to be diluted at least **1:10** with SAMPLEBUF (sample dilution buffer).

Tissue extract

- Pulverize about 200 mg of deep frozen tissue sample in a pre-frozen shaking holder of a micro-dismembrator (30 sec/1500 rpm).
- Homogenize the powder in 1 ml of phosphate buffer (0.14 M NaCl, 2.6 mM KCl, 8 mM Na₂HPO₄, 1.4 mM KH₂PO₄, 1 % Triton-X 100, pH 7.4). After ultracentrifugation (1 hour at 100,000 x g), the protein concentration should be determined in the supernatant by the commercially available Pierce-BCA or Peterson-Lowry Protein Assay.
- Use the supernatant for measurement in the assay.

Assay Procedure

Principle of the test

The assay utilizes the “sandwich” technique with two selected polyclonal antibodies that bind to human Realxin.

Assay standards, controls and pre-diluted patient samples which are assayed for human Relaxin are added into the wells of a microplate coated with a high affine polyclonal anti-human Relaxin antibody. During the first incubation step, Relaxin is bound by the immobilized antibody. Then a detection antibody, biotin-labeled anti Relaxin, is added. Afterwards a peroxidase-conjugate is added into each microtiter well and a “sandwich” of capture antibody - human Relaxin - detection antibody- peroxidaseconjugate is formed. Tetramethylbenzidine (TMB) is used as peroxidise substrate. Finally, an acidic stop solution is added to terminate the reaction.

The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of Realxin. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standard. Relaxin present in the patient samples is determined directly from this curve.

Principle of the test

- Do not interchange different lot numbers of any kit component within the same assay.
- Quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. PromoCell GmbH can therefore not be held responsible for any damage resulting from wrong use.
- The assay should always be performed according the enclosed manual.

Test procedure

1. Prior to use in the assay allow all reagents and samples to come to room temperature (18-26 °C) and mix well
2. Mark the positions of **STD** (Standards), **CTRL1,CTRL2** (control 1 and 2) and **SAMPLE*** (Sample) on a protocol sheet
3. Take microtiter strips out of the kit. Store unused strips covered at 2-8° C. Strips are stable until the expiry date stated on the label
4. Wash each well **5 times by dispensing 250 µl of diluted wash buffer** into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper
5. Add **100 µl** of **STD** (Standards), **CTRL1, CTRL2** (control 1 and 2) and **SAMPLE** (Sample) in duplicate into respective well. Use **SAMPLEBUF** (sample dilution buffer) as STD 0 pg/ml.
6. Cover the plate tightly and **incubate over night (16 - 22 hours) at 4 - 8 °C**
7. Discard the contents of each well. Wash each well **5 times by dispensing 250 µl of diluted wash buffer** into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper
8. Add **100 µl AB** (detection antibody, biotinylated) into each well
9. Cover the plate tightly and **incubate for 2 hours at 4 - 8 °C**
10. Discard the contents of each well. Wash each well **5 times by dispensing 250 µl of diluted wash buffer** into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper
11. Add **100 µl CONJ** (conjugate, streptavidin peroxidase-labeled a) into each well

12. Cover the plate tightly and **incubate for 1 hour at 4 - 8 °C**
13. Discard the contents of each well. Wash each well **5 times by dispensing 250 µl of diluted wash buffer** into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper
14. Add **100 µl of SUB** (substrate) into each well
15. Incubate for **20 - 30 minutes at room temperature** (18-26°C) in the dark*
16. Add **50 µl of STOP** (stop solution) into each well, mix thoroughly
17. Determine absorption **immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference

Results

The following algorithms can be used alternatively to calculate the results. We recommend using the "4-Parameter-algorithm".

1. 4-Parameter-algorithm
It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero calibrator has to be specified with a value smaller than 1 (e. g. 0.01).
2. Point-to-point-calculation
We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.
3. Spline-algorithm
We recommend for the optical density a linear ordinate and for the concentration a logarithmic abscissa. When using a

logarithmic abscissa, the zero calibrator has to be specified with a value smaller than 1 (e. g. 0.01).

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

Serum/plasma samples

For the calculation of the Relaxin concentration in **plasma/serum** the result has to be multiplied by **3**.

Urine samples

For the calculation of the Relaxin concentration in **urine** samples, the result has to be multiplied by **4**.

Seminal plasma samples

For the calculation of the Relaxin concentration in **seminal plasma** the result has to be multiplied by **10**.

Tissue extract

To obtain the concentration of the samples multiply the estimated values by the used dilution factor.

Limitations

Samples with Relaxin levels greater than the highest standard value should be further diluted with SAMPLEBUF (sample dilution buffer) and re-assayed.

Quality Control

PromoCell GmbH recommends the use of commercial control samples for internal quality control if available.

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Expected values

Normal ranges

We recommend each laboratory to establish its own norm concentration range.

Performance Characteristics

Precision and reproducibility

Intra-Assay (n=20)

Sample	Relaxin [pg/ml]	VC [%]
1	23.9	5.1
2	66.0	5.2

Inter-Assay (n=20)

Sample	Relaxin [pg/ml]	VC [%]
1	28.0	4.9
2	42.0	7.9

Cross reactivity

No cross reactivity with Insulin was observed.

Sensitivity

The sensitivity was set as B0 + 2SD. The zero-standard was measured 20 times.

Sample	Relaxin mean value [OD]	Standard variation (SD)	Detection limit [pg/ml]
1	0.04	0.006	0.5

Linearity

Two patient samples were diluted with SAMPLEBUF (sample dilution buffer) and analyzed. The results are shown below:

n= 2

Sample	Dilution	Expected [pg/ml]	Measured [pg/ml]
A	1:5	24.7	25.2
	1:6	20.6	21.0
	1:7	17.7	18.5
B	1:5	24.4	23.1
	1:6	20.4	20.0
	1:7	17.5	17.4

Precautions

- For *in vitro* diagnostic use only.
- The quality control guidelines should be followed.
- Human material used in the kit components was tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Reagents of the kit package contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. The substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water.

Technical Hints

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Substrate solution should remain colorless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.

General Notes on the Test and Test Procedure

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- Guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. PromoCell GmbH can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days after receipt of the product. The product shall be send to PromoCell GmbH together with a written complaint.

References

1. Armbruster et al. (2001) Eur J Med Res 6:1-9
2. Armbruster et al. (2001) Proceed third Intern Conference on Relaxin & Related Peptides, 2-27 October 2000, Broome, Australia, 273-274. Netherlands, Kluwer Academic Publishers. 2-10-200
3. Bani D (1997) Gen. Pharmac. 28:13-22
4. Bani D et al. (1998) A. J. Pathology 152:1367-1375
5. Nistri S et al. (2003) FASEB J 17 (14) 2109-2111
6. Dschietzig R, Stangl K (2002) CMLS 59: 1-13 (Review)
7. Dschietzig et al. (2004) Abstract of Fourth Intern Conference on Relaxin & Related Peptides, September 5-10, Jackson Hole, USA
8. Hocher B et al. (2004) Circulation 109: 2266-2268

Ordering Information

Product Name	Product Description	Size	Catalog Number
Relaxin ELISA Kit, human	Human Relaxin ELISA Kit	96 Tests	PK-EL-K9210

***For in vitro research use only.
Not for diagnostic or therapeutic procedures.***

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