Arbeitsanleitung / Manual

Relaxin ELISA

Zur in-vitro-Bestimmung von Relaxin in Serum, Plasma, Urin, Seminalplasma und Gewebe

For the in vitro determination of relaxin in serum, plasma, urine, seminal plasma and tissue

Gültig ab / Valid from 2022-05-25

REF **K 9210**

Σ 96

+2 °C +8 °C

IVD

CE

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

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1. INTENDED USE

This Immundiagnostik assay is an enzyme immunoassay intended for the quantitative determination of relaxin in serum, plasma, urine, seminal plasma and tissue samples. For *in vitro* diagnostic use only.

2. 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 recognised. 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

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 9210	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	1 x 100 ml
K 9210	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 1 vial
K 9210	AB	Detection antibody concentrate, biotin labelled rabbit anti-Relaxin	1 x 1 vial
K 9210	STD	Standards, lyophilised (0; 3.1; 9.3; 28; 83; 250 pg/ml)	2 x 6 vials
K 9210	CTRL1	Control, lyophilised (see specification for range)	2 x 1 vial
K 9210	CTRL2	Control, lyophilised (see specification for range)	2 x 1 vial
K 9210	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 50 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1 000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

Only for tissue extraction

- Micro-dismembrator
- Ultra-centrifuge, 100 000 *g*

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** 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 use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. Before use, the STD and CTRL have to be reconstituted with **500 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **Standards and controls** (reconstituted STD and CTRL) **can be stored at 2–8 °C for 4 weeks.**
- **Preparation of the detection antibody:** Before use, the **detection antibody concentrate (AB)** has to be diluted **1:1 001** 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. **Detection antibody** (1:1 001 diluted AB) **is not stable and cannot be stored.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:1 001** in **wash buffer** (10 µl CONJ + 10 ml wash buffer). The **CONJ** is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:1 001 diluted CONJ) **is not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

Serum, plasma, urine, seminal plasma and tissue can be stored at -20 °C.

Sample preparation

Serum and plasma

EDTA plasma or serum samples must be diluted at least **1:3** before performing the assay,

e. g. **100 µl** sample + **200 µl** dilution buffer (SAMPLEBUF), mix well.

For testing in duplicates, pipet **2 x 100 µl** of each prepared sample per well.

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 (Immundiagnostik Catalog number K 9212) 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 must be diluted at least **1:4** before performing the assay, e. g. **100 µl** sample + **300 µl** dilution buffer (SAMPLEBUF), mix well.

For testing in duplicates, pipet **2 x 100 µl** of each prepared sample per well.

Seminal plasma

Seminal plasma must be diluted at least **1:10** before performing the assay, e.g. **10 µl** sample + **1 990 µl** dilution buffer (SAMPLEBUF), mix well.

For testing in duplicates, pipet **2 x 100 µl** of each prepared sample per well.

Tissue extract

- Pulverise about 200 mg of deep frozen tissue sample in a pre-frozen shaking holder of a micro-dismembrator (30 s/1 500 rpm).
- Homogenise the powder in 1 ml of phosphate buffer(0,14 M NaCl, 2.6 mM KCl, 8 mM Na_2HPO_4, 1.4 mM KH_2PO_4, 1 % Triton-X 100, pH 7.4). After ultra-centrifugation (1 h/100 000 *g*), the protein concentration should be determined in the supernatant by the commercially available Pierce-BCA or Peterson-Lowry Protein Assay.
- For testing in duplicates, pipet **2 x 100 µl** of each supernatant per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of relaxin. The assay utilises 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 immobilised antibody. Then a detection antibody, biotin-labelled anti Relaxin, is added. Afterwards a peroxidase-conjugate is added into each microtiter well and a "sandwich" of capture antibody - human Relaxin - detection antibody–peroxidase-conjugate is formed. Tetramethylbenzidine (TMB) is used as peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction. The colour changes from blue to yellow. The intensity of the yellow colour is directly proportional to the concentration of Relaxin. 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.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet. We recommend a pretreatment of plasma and serum samples with the Immundiagnostik Anti Interference Reagent (Catalog number K 9212) prior to analysis.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until the expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	Wash each well **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
2.	Add each **100 µl standards/controls/samples** into the respective wells.

3.	Cover the strips and incubate for **over night (16-22 hours)** at 2–8 °C.
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
5.	Add **100 µl detection antibody** (diluted AB) in each well.
6.	Cover the strips and incubate for **2 hours at 2–8 °C**.
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
8.	Add **100 µl conjugate** (diluted CONJ) in each well.
9.	Cover the strips and incubate for **1 hour at 2–8 °C**.
10.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
11.	Add **100 µl substrate** (SUB) in each well.
12.	Incubate for **20–30 minutes*** at room temperature (15–30 °C) in the dark.
13.	Add **50 µl stop solution** (STOP) and mix well.
14.	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.

* The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. 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 standard must be specified with a value less than 1 (e.g. 0.001).

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 a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Serum and plasma samples

The obtained results have to be multiplied with the **dilution factor of 3** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

Urine samples

The obtained results have to be multiplied with the **dilution factor of 4** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

Seminal plasma samples

The obtained results have to be multiplied with the **dilution factor of 10** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

Tissue extract

The obtained results have to be multiplied with the **used dilution factor** to get the actual concentrations.

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

Analytical sensitivity × sample dilution factor to be used

Analytical sensitivity see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed 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.

Reference range

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 20)

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

Inter-Assay (n = 20)

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

Analytical Sensitivity

The zero standard was measured 20 times. The detection limit was set as B_0 + 2 SD and estimated to be 0.5 pg/ml.

Dilution recovery

Two patient samples were diluted and analysed. The results are shown below (n = 2):

Sample	Dilution	Relaxin expected [pg/ml]	Relaxin 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

Specificity

No cross reactivity to insulin was observed.

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Human materials used in kit components were 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.

- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are harmful to health and the environment. Substrates for enzymatic color reactions can also cause skin and/or respiratory irritation. Any contact with the substances should be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact. **Warning:** Causes serious eye irritation. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical advice/attention.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The 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. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

1. Armbruster et al. (2001) *Eur J Med Res* **6**:1-9
2. Armbruster et al. (2001) *Proceed third Intern Conference on Relaxin & Relates 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. Patholoy* **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

Used symbols:


Temperature limitation

Catalogue number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet


Irritant

Immundiagnostik AG

Stubenwald-Allee 8a
64625 Bensheim, Germany

Tel.: +49 6251 70190-0
Fax: +49 6251 70190-363

info@immundiagnostik.com
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