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R&D SYSTEMS

Quantikine™ ELISA

Human Corin Immunoassay

Catalog Number DCRN00

For the quantitative determination of human Corin concentrations in cell culture supernates, tissue homogenates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

Human Corin is a 1042 amino acid glycoprotein of approximately 150 kDa that belongs to the type II transmembrane multidomain protease (TTSP) family (1, 2). Its primary activity is its ability to activate atrial natriuretic peptide (ANP) by cleaving its pro-peptides (3, 4). It is also capable of cleaving brain-type, but not C-type, natriuretic peptide (3). Corin contains an N-terminal short cytoplasmic sequence followed by a transmembrane domain and the extracellular domain (ECD). The transmembrane domain is not needed for protease activity, but membrane localization of Corin on cardiac myocytes is thought to keep Corin near the site of pro-ANP release (5). The ECD contains two frizzled-like cysteine-rich domains, eight low density lipoprotein receptor-like (LDLR) domains, a scavenger receptor domain, and the serine protease catalytic domain (1, 2). Corin is the first TTSP member identified that contains frizzled-like domains, which are more commonly found in Wnt-interacting proteins (1). Not much is known about the function of most Corin domains, although the frizzled domains and the first four LDLR domains have been shown to influence protease activity (6-8). Cleavage at a site N-terminal to the catalytic domain of Corin is affected by glycosylation status and is required for activation of the protease (5, 6, 9). The cleaved portion remains tethered to the remainder of the protein via a disulfide linkage.

Corin is primarily expressed in the heart where it influences blood pressure regulation via ANP (1, 2). When hypertension induces stretching of cardiac myocytes, pro-ANP is released from storage in myocyte dense granules. The released pro-ANP is then cleaved by the Corin protease domain. The activated ANP induces excretion of water and sodium by the kidney, thus reducing blood volume and lowering blood pressure (1). Genetic deletion of Corin produces mice with hypertension and cardiac hypertrophy that is enhanced by a high-salt diet, consistent with a measured lack of ANP activation (10). In humans, an allele common in African-Americans is associated with hypertension and cardiac hypertrophy, symptoms of ANP hypoactivity and indicators of Corin hypoactivity (7, 8). This allele contains two alternate amino acids within the second frizzled-like domain (Q568P and T555I). Pregnant mice that lack Corin show hypertension reminiscent of human gestational hypertension (10). This is consistent with expression of Corin in the uterus during pregnancy, where it is thought to regulate the response of ANP to increased blood volume (10). Corin expression has also been detected in developing bone and kidney and also in some cancer cells such as small cell lung cancer (SCLC) cells (2, 11). It is thought to contribute to the pathogenesis of the syndrome of inappropriate anti-diuretic hormone in SCLC and some other cancers (11). Little is known about soluble forms of Corin that are present in the circulation.

The Quantikine™ Human Corin Immunoassay is a 4.5 hour solid phase ELISA designed to measure human Corin in cell culture supernates, tissue homogenates, serum, and plasma. It contains a recombinant human Corin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Corin showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human Corin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Corin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Corin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Corin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Corin bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine™ Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Corin Microplate	893273	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Corin.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human Corin Standard	893275	Recombinant human Corin in a buffered protein solution with preservatives, lyophilized. *Refer to the vial label for the reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C.* Avoid repeated freeze-thaw cycles.
Human Corin Conjugate	893274	12.5 mL of polyclonal antibody specific for human Corin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-41	895514	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-1	895163	21 mL of buffered animal serum with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 500 mL graduated cylinder
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples
- Human Corin Controls (optional; R&D Systems®, Catalog # QC67)

SUPPLIES REQUIRED FOR TISSUE HOMOGENATE SAMPLES

- Extraction Buffer Concentrate (R&D Systems, Catalog # 895287)

PRECAUTIONS

Calibrator Diluent RD6-1 contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Homogenates - Preparation of tissue homogenates will vary depending on the tissue type. See Sample Values for preparation of tissue used to generate sample value data.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Hemolyzed samples or samples with high protein levels are not suitable for use in this assay.

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD6-1.

REAGENT PREPARATION

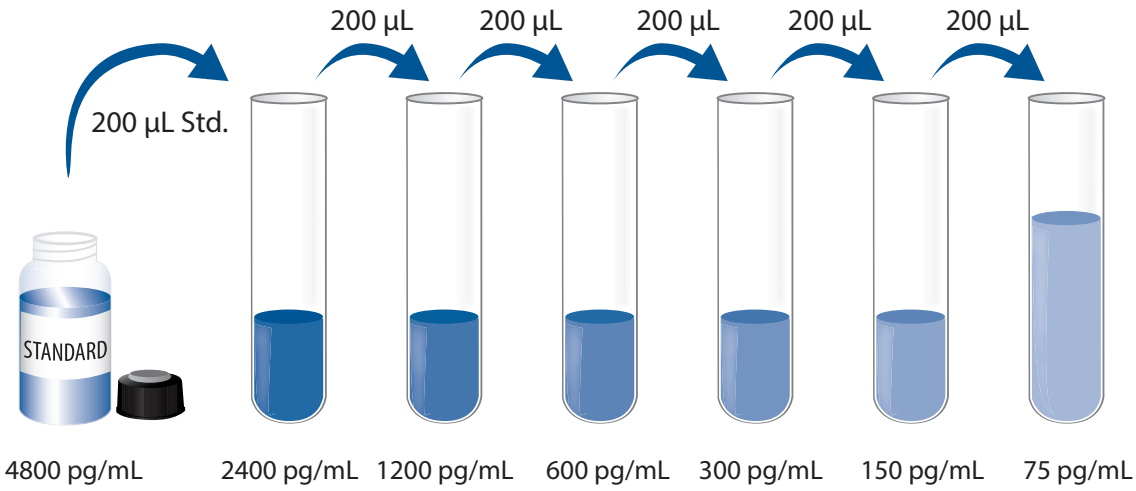
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Human Corin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Corin Standard with Calibrator Diluent RD6-1. This reconstitution produces a stock solution of 4800 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD6-1 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4800 pg/mL standard serves as the high standard. The Calibrator Diluent RD6-1 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-41 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Human Corin Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

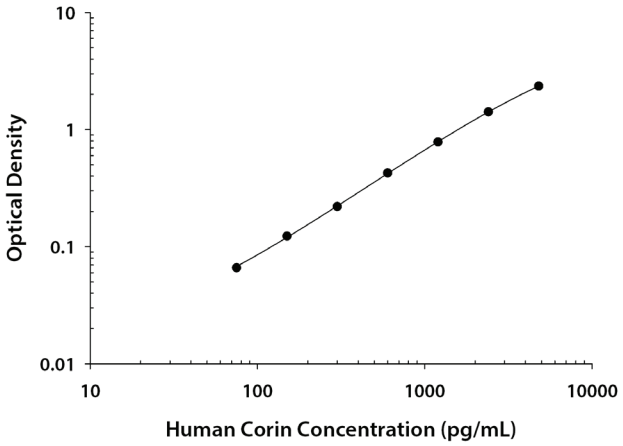
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Corin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.080 0.084	0.082	—
75	0.147 0.148	0.148	0.066
150	0.205 0.205	0.205	0.123
300	0.301 0.303	0.302	0.220
600	0.505 0.510	0.508	0.426
1200	0.860 0.869	0.865	0.783
2400	1.496 1.497	1.497	1.415
4800	2.422 2.441	2.432	2.350

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	618	1327	2794	660	1378	2819
Standard deviation	16.9	35.6	69.6	41.4	61.3	106
CV (%)	2.7	2.7	2.5	6.3	4.4	3.8

RECOVERY

The recovery of human Corin spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=6)	96	88-101%
Serum* (n=6)	94	89-100%
Heparin plasma* (n=6)	97	88-107%
EDTA plasma* (n=6)	94	87-99%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Corin were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Tissue homogenates (n=2)	Serum* (n=6)	EDTA plasma* (n=6)	Heparin plasma* (n=6)
1:2	Average % of Expected	100	101	100	100	100
	Range (%)	98-100	99-102	98-101	99-102	99-101
1:4	Average % of Expected	96	102	97	968	98
	Range (%)	96-97	101-103	96-99	95-105	93-102
1:8	Average % of Expected	96	98	94	92	93
	Range (%)	93-101	98-98	90-96	86-98	87-101
1:16	Average % of Expected	95	93	88	90	92
	Range (%)	92-100	93-94	———	———	———

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Sixty-six assays were evaluated and the minimum detectable dose (MDD) of human Corin ranged from 1.60-23.7 pg/mL. The mean MDD was 5.64 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human Corin produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Corin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=38)	1359	578-3138	626
Heparin plasma (n=38)	1318	568-3058	572
EDTA plasma (n=38)	1182	489-3020	611

Note: *Serum and plasma samples were approximately 80% higher in male versus female samples.*

Cell Culture Supernates/Cell Lysates - The following cell lines were assayed for natural human Corin. No detectable levels were observed.

Cell Lines	Cell Type	Growth Conditions
COLO 205	Human colorectal adenocarcinoma	RPMI + 10% FBS for 4 days
U937	Human histiocytic lymphoma	RPMI + 10% FBS for 1 and 3 days
HEK293	Human embryonic kidney fibroblasts	DMEM + 10% FBS for 14 days
N1186	Human T cell line	RPMI + 10% FBS for 10 days
HT-29	Human colon adenocarcinoma	McCoy's 5a + 10% FBS for 4 days
NCI-H378	Human small cell lung carcinoma	RPMI + 10% FBS for 4 days
NCI-H128	Human small cell lung carcinoma	RPMI + 20% FBS for 6 days

Tissue Homogenates - Human heart tissue (1.96 grams) was homogenized with a Dounce homogenizer in 11 mL of Extraction Buffer (diluted 1:5 with deionized or distilled water). The homogenate was then subjected to two freeze/thaw cycles at ≤ -20 °C. The resulting lysate was centrifuged to remove debris and filtered through a 0.2 mm filter. The lysate sample contained 4.82 mg/mL of total protein. When assayed for human Corin, the lysate sample measured 51.8 ng/mL (10.7 ng of Corin per mg of total protein).

SPECIFICITY

This assay recognizes natural and recombinant human Corin.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Corin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ANP
Enterokinase
HAT
Hepsin
LDLR
Matriptase (aa 615-855)
Serpin A1
Serpin A3
Serpin A5
Serpin C1
Serpin F1/PEDF
Spinesin

REFERENCES

1. Wu, Q. (2007) Front. Biosci. **12**:4179.
2. Yan, W. *et al.* (1999) J. Biol. Chem. **274**:14926.
3. Yan, W. *et al.* (2000) Proc. Natl. Acad. Sci. USA **97**:8525.
4. Wu, F. *et al.* (2002) J. Biol. Chem. **277**:16900.
5. Knappe, S. *et al.* (2003) J. Biol. Chem. **278**:52363.
6. Knappe, S. *et al.* (2004) J. Biol. Chem. **279**:34464.
7. Dries, D.L. *et al.* (2005) Circulation **112**:2403.
8. Rame, J.E. *et al.* (2007) Hypertension **49**:857.
9. Liao, X. *et al.* (2007) J. Biol. Chem. **282**:27728.
10. Chan, J.C.Y. *et al.* (2005) Proc. Natl. Acad. Sci. USA **102**:785.
11. Wu, F. and Q. Wu (2003) Can. Res. **63**:8318.12.