**Décembre 2016 – Modèle 20**

**Cisbio Bioassays**
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# RENIN III GENERATION

REF *RENINE* Σ 100 IVD CE 2°C 8°C

| **Trousse pour le dosage radioimmunométrique de la RENINE active dans le plasma humain**<br>**Pour diagnostic In Vitro** | **Radioimmunometric assay kit for the quantitative determination of active RENIN in human plasma**<br>**For In Vitro diagnostic use** | **Immunoradiometrischer Test zur Bestimmung des aktiven RENIN in Humanplasma**<br>**Zur In Vitro Diagnostik** |
|---|---|---|
| La trousse contient : | Kit content: | Inhalt des Kits: |
| Tubes revêtus 2 x 50<br>Traceur ≤ **360 kBq** 1 x 11 mL<br>Calibrateur (CAL 0) (diluent) 1 x 7 mL<br>Calibrateurs (CAL1 – CAL5) 5 x qsp 3 mL<br>Réactif de lavage 1 x 25 mL<br>Contrôle 1 x qsp 2 mL<br>Sachet plastique 1<br>Notice d'utilisation 1 | Coated tubes 2 x 50<br>Tracer ≤ **360 kBq** 1 x 11 mL<br>Calibrator (CAL 0) (diluent) 1 x 7 mL<br>Calibrators (CAL1 – CAL5) 5 x qs 3 mL<br>Wash reagent 1 x 25 mL<br>Control 1 x qs 2 mL<br>Plastic bag 1<br>Instruction for use 1 | Teströhrchen beschichtet 2 x 50<br>Tracer ≤ **360 kBq** 1 x 11 mL<br>Kalibrator (CAL 0) (Verdünner) 1 x 7 mL<br>Kalibratoren (CAL1 – CAL5) 5 x q.s 3 mL<br>Waschreagens 1 x 25 mL<br>Kontrolle 1 x q.s 2 mL<br>Plastikbeutel 1<br>Arbeitsanleitung 1 |
| **Attention :** Certains réactifs contiennent de l'azoture de sodium | **Warning:** Some reagents contain sodium azide | **Achtung:** Einige Reagenzien enthalten Natriumazid |
| **Kit per il dosaggio radioimmunologico della RENINA attiva nel sangue umano**<br>**Per uso diagnostico In Vitro** | **Equipo radioinmunométrico para la determinación cuantitativa de la RENINA activa en plasma humano.**<br>**Para diagnóstico in vitro** | **Kit de radioimunoensaio para a determinação quantitativa da RENINA activa no plasma humano**<br>**Para diagnóstico *In Vitro*** |
| Contenuto del kit: | Contenido del equipo: | O kit contém: |
| Provette coattate 2 x 50<br>Tracciante ≤ **360 kBq** 1 x 11 mL<br>Calibratore (CAL 0) (diluente) 1 x 7 mL<br>Calibratori (CAL1 – CAL5) 5 x q.b a 3 mL<br>Reagente di lavaggio 1 x 25 mL<br>Controllo 1 x q.b a 2 mL<br>Sacchetto di plastica 1<br>Istruzioni per l'uso 1 | Tubos recubiertos 2 x 50<br>Trazador ≤ **360 kBq** 1 x 11 mL<br>Calibrador (CAL 0) (dluyente) 1 x 7 mL<br>Calibradores (CAL1 – CAL5) 5 x csp 3 mL<br>Reactivo de lavado 1 x 25 mL<br>Control 1 x csp 2 mL<br>Bolsa de plástico 1<br>Instrucciones de empleo 1 | Tubos revestidos 2 x 50<br>Traçador ≤ **360 kBq** 1 x 11 mL<br>Calibrador (CAL 0) (diluente) 1 x 7 mL<br>Calibradores (CAL1 – CAL5) 5 x q.b.p. 3 mL<br>Reagente de lavagem 1 x 25 mL<br>Controlo 1 x q.b.p. 2 mL<br>Saqueta plástica 1<br>Instruções de utilização 1 |
| **Attenzione:** Alcuni reagenti contengono sodio azide | **Precauciones:** Algunos reactivos contienen azida sódica | **Atenção:** Certos reagentes contêm azida de sódio. |

| | FRA | ENG | DEU | ITA | SPA | POR | SWE | DAN | NOR | ELL | CES | POL | SVK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Explication des symboles** | **Explanation of symbols** | **Erläuterung der Sumbole** | **Spiegazione dei simboli** | **Significado de los simbolos** | **Significadodos simbolos** | **Symbolförklaring** | **Symbolforklaring** | **Forklaring av symbolene** | **Επεξήγηση των συμβόλων** | **Wyjaśnienie symboli** | **Vysvětlení symbolů** | **Vysvetlenie symbolov** |
| CE | Conforme aux normes européennes | European conformity | CE-Konformitäts-kennzeichnung | Conformita europea | Conformidad europea | Conformidad com as normas europeias | Förenlig med europeiska normer | I overensstemmelse med de europæiske standarder | Europeisk konformitet | Συμμόρφωση με τα ευρωπαϊκά πρότυπα | Zgodne z normami europejskimi | Evropská shody | Európska zhoda |
| | T° limite de stockage | torage limitation temperature | Limitierung der Lagertemperatur | Limiti per la temperatura di conservazione | Limites de temperatura de almacenamiento | Limite da temperatura de armazenagem | T°-gräns vid förvaring | T° grænse for opbevaring | Lagertemperatur begrensning | Όρια θερμοκρασίας αποθήκευσης | Graniczna temperatura przechowywania | Mezní teplota skladování | skladovania teplota |
| LOT | N° de lot | Batch code | Chargencode | codice lotto | Código de lote | Lote | Lotnr. | Lot nr. | Porsjoner kode | Κωδικός παρτίδας | Numer partii | Č. šarže | Kód šarže |
| | Utiliser jusqu'au | Use by | Verwendbar bis | utilizzare entro | Consumir antes de | Utilizado por | Används senast | Udløbsdato | Bruk ved | Χρήση έως | Zużyć do | Použitelné do | Použiteľné do |
| | Consulter la notice d'utilisati | Consult operating instructions | Das Handbuch zu Rate ziehen | consultare le istruzioni per l'USO | Consultar las instrucciones de manejo o funcionamiento | Consulte o manual de operações | Läs bruksanvisningen | Se brugsvejledningen | Konsulter driftsinstrukser | Συμβουλευτείτε τις οδηγίες χρήσης | Patrz dołączona ulotka | Přečtěte si návod k použití | Prečítajte si návod na použitie |
| IVD | Diagnostic In Vitro | In Vitro Diagnostic device | In-VitroDiagnostische Anwendung | Dispositivo Diagnostico In Vitro | Dispositivo de diagnóstico In Vitro | Dispositivo de diagnostico In Vitro | *In vitro*-diagnos | In vitro diagnose | In Vitro Diagnose innretning | In vitro διαγνωστική συσκευή | Diagnostyka In Vitro | Diagnostika in vitro | In vitro diagnostická pomôcka |
| | Fabriqué par | Manufactured by | Hergestellt von | Prodotto da | Fabricado por | Fabricado por | Tillverkad av | Fremstillet af | Produsere ved | Κατασκευάζεται από | Wyprodukowane przez | Vyrobil | Výrobca |
| REF | Référence | Catalogue number | Katalog Nr. | N. catalogo | Número de catálogo | Número do catalogo | Referens | Reference | Katalogiser antall | Αριθμός καταλόγου | Wzorzec | Reference | Katalógové číslo |
| Σ 100 | Nombre de tubes | Number of determinations | Anzahl der Bestimmungen | Numero di determinazioni | Número de determinaciones | Número de determinações | Antal rör | Antal glas | Antall determinations | Αριθμός προσδιορισμών | Liczba probówek | Počet zkumavek | Počet stanovení |
| CT | Tubes revêtus | Coated tubes | beschichtete Röhrchen | Provette coattate | Tubos recubiertos | Tubos adsorvidos | Belagda rör | Beklædte glas | Belagt rør | επιστρωμένα σωληνάρια | Probówki powlekane | potažené zkumavky | Potiahnuté skúmavky |
| TRACER | Traceur radioactif | Radioactive tracer | Radioactiver Tracer | Tracciante radioattivo | Trazador radiactivo | Marcador radioativo | Radioaktiv tracer | Radioaktivt sporstof | Radioaktiv tracer | Ραδιενεργός ιχνηθέτης | Znacznik radioaktywny | Tracer | Rádioaktívny značkovač |
| CAL | Calibrateur | Calibrator | Kalibrator | Calibratore | Calibrador | Calibrador | Kalibrator | Kalibrator | Calibrator | Βαθμονομητής | Kalibrator | Kalibrátor | Kalibrátor |
| CONTROL | Contrôle | Control | Kontrolle | Controllo | Control | Controle | Kontroll | Kontrol | Styring | Ορός ελέγχου | Kontrola | Kontrola | Kontrolný roztok |
| WASH | Solution de lavage | Wash solution | Waschlotion | Soluzione di lavaggio | Solución de lavado | Solução de lavagem | Tvättlösning | Vaskeopløsning | Vaskløsning | ΔΙΑΛΥΜΑ ΠΛΥΣΗΣ | roztwór do płukania | promývací reagencie | Oplachovací roztok |

# MISE A JOUR / UPDATING

CE

**RENINE**
**Cisbio Bioassays - Décembre 2016 - Modèle 20**

**FRA** **Modifications par rapport à la version précédente :**
Nouveau logo / 1. Ajout de « La trousse est destinée à un usage professionnel » / 6. Enlever précision (±1%) / 11. Mise à jour du paragraphe – Mise à jour de la courbe de calibration – Information mode de lissage.

**ENG** **Changes from the previous version:**
New logo / 1. "The kit is intended for professional use"adding / 6. remove ±1% (precision) / 11. § updated – Calibration curve updated - Information on fitting model.

**DEU** **Änderungen gegenüber der Vorgängerversion:**
Neues Logo /1. „Das Kit ist für den professionellen Gebrauch vorgesehen" hinzufügen / 6. ±1% (Präzision) entfernen / 11. Absatz aktualisiert - Kalibrationskurve aktualisiert - Informationen zum Funktionsmodell.

**ITA** **Modifiche rispetto alla versione precedente:**
Nuovo logo / 1. Aggiunto "Il kit è destinato all'uso professionale" / 6. Eliminato ±1% (precisione) / 11. § aggiornato - Aggiornata la curva di calibrazione - informazioni sul modello di fitting.

**SPA** **Cambios desde la versión anterior:**
Nuevo logotipo / 1. Se ha añadido "El kit está destinado a uso profesional" / 6. Borrar ±1% (precisión) / 11. Párrafo actualizado –
curva de calibración actualizada - información sobre el modelo de ajuste.

**POR** **Alterações em relação à versão anterior:**
Novo logótipo / 1. „O kit destina-se a utilização profissional" adicionando /6. remoção ±1% precisão / 11. § atualizado –– curva de calibração atualizada - Informação sobre o modelo de ajuste

**SWE** **Ändringar från den föregående versionen:**
Ny logotyp /1. "Setet är avsett för yrkesmässigt bruk" läggs till / 6. avlägsna ± 1% (precision) /11. § uppdaterad-– uppdaterad kalibreringskurva - Information om anpassningsmodell .

**DAN** **Ændringer fra den tidligere version:**
Nyt logo / 1. Sættet er beregnet til professionel brug" tilføjet / 6. Fjernet ±1% præcision / 11. § opdateret – kalibreringskurve opdateret - Information om tilpasningsmodel

**NOR** **Endringer fra forrige versjon:**
Ny logo / 1. „Kitet er beregnet på profesjonell bruk» / 6 Fjern ± 1% presisjon / 11. § oppdatert – kalibratorkurve oppdatert - Informasjon om tilpasningsmodellen

**ELL** **Αλλαγές από την προηγούμενη έκδοση:**
Νέο λογότυπο / 1. Προσθήκη "Το κιτ προορίζεται για επαγγελματική χρήση"/ 6. αφαίρεση ± 1% (ακρίβεια) / 11. Ενημέρωση παραγράφου –– ενημέρωση της καμπύλης βαθμονόμησης - Πληροφορίες σχετικά με το μοντέλο προσαρμογής.

**POL** **Zmiany w stosunku do poprzedniej wersji:**
Nowe logo / 1. Dodanie „Zestaw jest przeznaczony do zastosowania profesjonalnego" / 6. usunąć z precyzją ±1% / 11. zaktualizowany § - aktualizacja krzywej kalibracji - Informacje na temat modelu dopasowywania

**CES** **Změny od předchozí verze:**
Nové logo / 1. Přidání „Souprava je určena pro profesionální použití" / 6. odstranění přesnosti ±1 % / 11. § aktualizováno - Informace o přizpůsobeném modelu – kalibrační křivka aktualizována

**SVK** **Zmeny oproti predchádzajúcej verzii:**
Nové logo / 1. Vloženie „Súprava je určená na profesionálne použitie"/ 6. Odstrániť presnosť ±1 %/ 11. § aktualizovaná - Informácie o modeli vyrovnania – aktualizovaná krivka kalibrácie.

## 1. INTENDED USE

RENIN III GENERATION is a radioimmunometric assay for the quantitative *in vitro diagnostic* measurement of the level of active renin in plasma. Renin measurements are used in the diagnosis and treatment of certain types of hypertension.
The kit is intended for professional use.

## 2. SUMMARY AND EXPLANATION

Renin is a proteolytic acidic enzyme produced and secreted by the juxtaglomerular cells. It cleaves angiotensinogen into angiotensin I (inactive), which ultimately leads to the production of angiotensin II (active).
Therefore, renin, which has a limitating effect on the production of angiotensin, is a key-factor in the regulation of arterial pressure and hydrosodic metabolism.
As most enzymes which act outside of the cells in which they are synthesized, renin exists in both inactive and active forms. Inactive renin (prorenin) which is found in plasma, amniotic fluid and in the kidney, can be activated in different ways (cryoactivation, acidification or partial proteolysis) which expose the active site of the enzyme. Inactive renin can account for up to 90 % of the total renin in the circulation.
However, it is the active renin which provides the medium through which biological activity takes place.
Now, human active renin is well known: it is a polypeptidic chain of 345 amino acids, with a molecular weight of about 40,000.
Angiotensinogen, the substrate of renin, is a liver protein from which angiotensin I is produced.
It must be noted that the concentration of angiotensinogen in circulation influences the level of plasmatic renin activity and so, ultimately, the level of angiotensin II. This shows the importance, for the synthesis of angiotensinogen, of those factors directly active at the liver level.
It has been proved that the in vivo hypertensive action of angiotensin I is due to its conversion into angiotensin II by a carboxypeptidase (converting enzyme). The converting enzyme is regulated by the glucocorticoids and the thyroid hormones. Angiotensin II is the major effector in the renin-angiotensin system, maintaining circulatory homeostasis through its direct effect on the smooth vascular muscle and on the stimulation of aldosterone, and by its stimulatory effect on the sympathetic nervous system.

In the kidney, angiotensin II is involved in the control of glomerular filtration and in renal blood flux. Renin is secreted by the kidneys in response to a reduction in the perfusion of the renal artery (intrarenal baroceptor), a reduction of distal tubular reabsorption of $Na^+$ (sodium leakage), a hypokaliemia or a B-adrenergic stimulation. In addition renin secretion is reduced (negative feed back) when there is a high plasmatic concentration of angiotensin II.

Renin assay is necessary in hypertensive patients and in the therapeutic follow up of high blood pressure. Renin should be measured:

- Whenever diastolic blood exceeds 110 mm Hg (to trace a hypertension of renal origin).
- Whenever there is a hypokaliemia (< 3.8 mmol/L): to try to find a secondary hyperaldosteronism or a primary hypermineralocorticism.
- Whenever the response to antihypertensive treatment is insufficient.
- In order to determine the functional character of a renal artery stenosis (by measurement of renin in the renal veins during acute inhibition of the converting enzyme).
- Whenever a cancer is linked to an increase in blood pressure (to look for ectopic production of renin).

## 3. PRINCIPLE

RENIN III GENERATION is an immunoradiometric (sandwich technique) with following characteristics:

- Its uses a pair of anti-renin Monoclonal Antibodies (MAb), selected on the basis of three very precise criteria: specificity, avidity and complementary.
- The first monoclonal antibody, coated on polystyrene tubes, specifically recognizes both the active and inactive forms of renin.
- The second monoclonal antibody labelled with 125 Iodine, specifically recognizes the active form of renin.

The assay involves the following steps:

1. Incubation of calibrator and unknown sera in the presence of an excess of the first insolubilized monoclonal antibody on the wall of polystyrene tubes and of an excess of the second 125 Iodine monoclonal antibody.
2. Washing for elimination of the free fraction and measurement of activity bound to the solid phase.
3. The amount of bound radioactivity of the bound complex is measured in a Gamma counter. Results of samples can be determined directly using the calibrator curve.
4. The antigen in the calibrators and control is of a recombinant origin.
5. The kit calibrators are calibrated against the international reference preparation: WHO: 68/356. (See chapter 5. Reagents provided).

## 4. WARNINGS AND PRECAUTIONS

1. For in-vitro diagnostic use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

3. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents. Avoid any microbial contamination of the reagents or of the water. Fully respect the incubation conditions and the washing instructions indicated.
4. Follow good laboratory practice and safety guidelines. Wear lab coats, and disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards. Do not pipette by mouth. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Avoid splashing.
5. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
6. This kit contains radioactive material, to be received, acquired, possessed and used by physicians, laboratories or hospitals only according to regulations and a specific license issued by the Nuclear Regulatory Commission or issued by a state with which the Nuclear Regulatory Commission has entered into an agreement for the exercise of regulatory authority.
7. Radioactive materials should be confined to specifically designated, regularly monitored areas in the laboratory, restricted to authorized personnel. Radioactive products must be stored in their original containers in a suitable area. A record of the reception and storage of radioactive products must be kept up to date. Use disposable labware and disposable absorbent bench covers. Always wear film budges, lab coats and disposable gloves. Wipe up all spills immediately, cleaning the contaminated area with a decontaminant and dispose the contaminated materials as radioactive waste. Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross- contamination of different isotopes. All radioactive waste disposal must be carried out according to the regulations in force
8. Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.
9. Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.
10. Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

## 5. REAGENTS PROVIDED

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

| REAGENTS | SYMBOLS | QUANTITY | STORAGE |
|---|---|---|---|
| **COATED TUBES**: ready for use.<br>Mouse monoclonal antibody anti human renin coated on the bottom of the tube. | **CT** | 2 packs of 50 tubes | 2-8°C until the expiry date.<br>After packaging opening, unused antibodies coated tubes must be stored in the plastic bag. |
| **ANTI-RENINE $^{125}$I**: ready for use.<br>Mouse anti-human active renin monoclonal antibody $^{125}$I in a Tris buffer pH 7.9 containing horse serum and 0.1 % sodium azide ($NaN_3$). One vial contains around **360 kBq** with a specific radioactivity of 925 kBq/µg so that 210 000 cpm can be obtained for 100 µL at the day of the labelling. | **TRACER** | 1 - 11 mL vial | 2-8°C until the expiry date. |
| **CALIBRATOR (CAL 0)**: ready for use.<br>Phosphate buffer pH 7.4 containing casein, 0.1 % sodium azide and a dye. | **CAL** | 1 - 7 mL vial | 2-8°C until the expiry date. |
| **CALIBRATORS (CAL 1 - CAL 5)**: lyophilized. (*)<br>Active renin diluted in S0, containing 0.1 % sodium azide.<br>2.5 - 5 -20 - 80 - 320 pg/mL.<br>Reconstitute with 3 mL of demineralised or distilled water.<br>After complete dissolution it must be carefully homogenized.<br>Avoid foam formation during homogenization. | **CAL** | 5 each qs 3 mL vials | 2-8°C until the expiry date.<br>After reconstitution, calibrators can be used within 4 hours at room temperature (18-25°C). Then, they can be kept 10 days at 2-8°C or aliquoted and kept frozen at –20°C for 6 weeks. (***) |
| **CONTROL**: lyophilized. (**)<br>Sample of human origin containing 0.1 % sodium azide.<br>Reconstitute with 2 mL of demineralised or distilled water.<br>After complete dissolution it must be carefully homogenized.<br>Avoid foam formation during homogenization. | **CONTROL** | 1 - qs 2 mL vial | 2-8°C until the expiry date.<br>After reconstitution, control sample must be aliquoted and rapidly kept frozen at –20°C. In this condition it is stable 6 weeks. (***) |

| | | | |
|---|---|---|---|
| **WASHING SOLUTION (R3)** :<br>Imidazole-buffer pH 7.4 with Tween 20 and sodium azide (0.1 %). Dilute the content in 975 mL of demineralised or distilled water.<br>Mix before use. | **WASH** | 1 -<br>25 mL vial | 2-8°C until the expiry date.<br>After reconstitution: 6 weeks at 2-8°C. |
| **PLASTIC BAG** | | 1 | |

(*) The values shown above are only target values: the true value of each calibrator is shown on its label.
The equivalence with respect to the international reference (WHO 68/356) was measured as 1.8 $10^{-6}$ with an uncertainty of +/- 10 %. (To obtain values expressed in International Units, it is necessary to multiply the results with the CISBIO kit by a factor of 1.8 $10^{-6}$ ).
(**) The acceptance range true values are printed on the vial label.
(***) Calibrators and control could be frozen and thawed once.

## 6. MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision micropipettes or similar with disposable tips, capable of dispensing 100 µL, 300 µL and 3 mL. Their calibration should be checked regularly.
2. Distilled or demineralized water.
3. Disposable round-bottom plastic test tubes (12 x 75 mm).
4. Rack for test tubes.
5. Graduated cylinder (1 Liter).
6. Parafilm®.
7. Circular horizontal shaker (400rpm).
8. Gamma scintillation Counter calibrated for 125 Iodine measurement.

## 7. STORAGE CONDITIONS

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding sections.

## 8. SPECIMEN COLLECTION AND PREPARATION

**The assay is performed directly on EDTA plasma.**
If the test is not run within 4 hours following sampling, samples must be aliquoted and stored deep frozen at - 20°C. Under these conditions samples can be stored 10 months. After thawing plasma must be shaken and carefully centrifuged to eliminate any trace of fibrin.

**Warning :**

***"Hemoglobin concentrations in excess of 1.25 mg/ml seriously interfere with measurements made with this assay. Do not use this assay on samples if slight hemolysis is suspected."***

Do not use severely lipemic samples.
Avoid successive freezing and thawing.
Do not store plasma at + 4°C as possible activation may occur.

**Dilutions**
Should elevated renin (> 320 pg/mL) levels be suspected, the calibrator 0 found in the kit is used for dilution.
It is recommended that disposable plastic tubes be used when carrying out dilutions.

## 9. ASSAY PROCEDURE

Do not mix reagents from different batches.
All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use.

Dispensing of the reagents into the tubes is also carried out at room temperature.

The assay requires the following groups of tubes : Tube T for the Total Activity, calibrator "0" group for the determination of non-specific binding, Calibrator groups to establish the calibrator curve, Control group for the control, Six groups for the samples to be assayed.

It is recommended to perform the assay in duplicate for calibrators, control and samples.

If necessary carefully centrifuged thawed samples.

A calibrator curve should be performed on each test occasion.

Observe the order in which reagents have to be added :

- Dispense 300 µL of calibrators, control or samples into the corresponding groups of tubes.
- Add 100 µL of tracer $^{125}$I into all tubes. Close tubes T.
- Mix gently. Cover with Parafilm®.
- Incubate for 3 hours at room temperature (18-25°C) under constant horizontal agitation.

Wash the coated tubes as follows :

- Discard the reactive medium by aspiration (or by inversion), except tubes T.
- Add 2.0 mL of washing solution (diluted to 1/40 in distilled or demineralised water) to each tube, except tubes T.
- Discard the washing solution by aspiration (or by inversion).
- Repeat the process twice.
- Then, leave the tubes to stand 2 minutes on the inverting position or aspirate the contents of the tubes as completely as possible. There must be no residual volume in the coated tubes after washing.

To obtain reliable and reproducible results, the different washing steps have to be correctly performed. The addition of the washing solution must be carried out with an efficient speed in order to create turbulences into the tubes.
Measure the remaining radioactivity bound to the tube with a gamma scintillation counter for 2 minutes.

## 10. QUALITY CONTROL

Good Laboratory Practices (GLP) require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended. The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP or other applicable federal, state and local calibrators/laws. All calibrators and kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated.
It is recommended to participate at appropriate quality assessment trials.

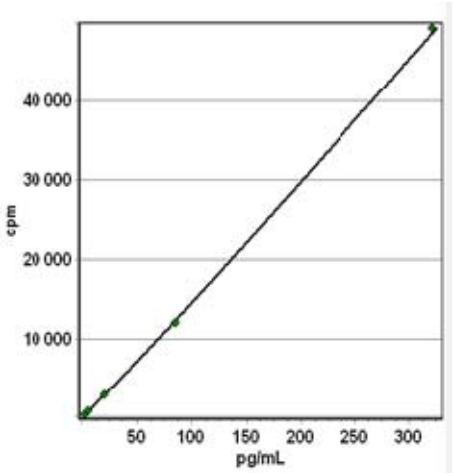
## 11. CALCULATION OF RESULTS

For each group of tubes, calculate the mean of the counts.
Plot the calibration curve, expressing the cpm of the calibrators according to their concentration.
Read the sample values directly off the curve, correcting using the dilution factor if necessary.

The linear regression mathematical fitting model is recommended for calibration curve. Other fitting model may give slightly different results.

**Typical calibrator curve** (example only) : these data must under no circumstances be substituted for results obtained in the laboratory.

| | Bound cpm | Concentration pg/mL |
|---|---|---|
| Total activity | 186 335 | |
| CAL 0 | 19 | 0.00 |
| CAL 1 | 333 | 2.60 |
| CAL 2 | 682 | 5.30 |
| CAL 3 | 2 798 | 20.60 |
| CAL 4 | 12 046 | 85.00 |
| CAL 5 | 49 394 | 320.00 |
| Control | 4 679 | 34.00 |




## 12. EXPECTED NORMAL VALUES

It is critically important to recognize that many factors (posture, age, sodium intake, menstrual cycle) can influence the active renin level. Thus, and with every diagnostic test, it is recommended that each laboratory establishes its normal range values according to its own population.
In order to determine the normal range of RENIN III GENERATION, 125 samples from fasting adult males and females, without estrogen-progesterone treatment, apparently healthy subjects ages 20 to 60 years were analyzed using the RENIN III GENERATION kit. The subjects were asked to perform 1 hour of activity in an upright position and then remain in a supine for 1 hour. At the end of each 1 hour cycle a blood sample was drawn and tested in the Renin Assay. The results are shown in the Table below.

| RESULTS | AGE | N | MEAN (pg/mL) | S.D. | Range |
|---|---|---|---|---|---|
| Upright | 20-40 | 50 | 13.10 | 6.84 | 5.1 - 38.7 |
| | 40-60 | 75 | 14.02 | 8.34 | 1.8 - 59.4 |
| Supine | 20-40 | 50 | 8.11 | 3.66 | 3.6 - 20.1 |
| | 40-60 | 75 | 6.18 | 3.42 | 1.1 - 20.2 |

## 13. LIMITATIONS OF PROCEDURE

1. Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results.
2. Do not extrapolate sample values beyond the last calibrator. Dilute the samples concerned and re-assay.
3. The significance of active renin measurements can be meaningfully interpreted only when the patient is studied under controlled conditions and with defined sodium balance.
4. Since a number of physiological factors can influence the renin secretion, conditions under which samples are collected must be carefully controlled :
   a. the patient must not have taken any antihypertensive medication for 8 days.
   b. posture must be controlled : he must have been lying down for more than one hour or upright for more than one hour.
   c. sodium content in the diet must be known and eventually verified by the measurement of natriuria over a period of 24 hours (60 to 200 mEq/24 h).
5. It must be known that physiological factors affect renin secretion :
   a. both levels of inactive and active renin increase during pregnancy,
   b. menstrual cycle : increase of the level on the second phase of the cycle (sampling is to be done if possible during the first phase),
   c. active renin level decreases with age,
   d. Nycthemeral cycle affects also the concentration: sampling is to be done between 7 AM and 10 AM if possible.
6. It must be also noted that various medications could affect the renin secretion :
   a. Diuretics, inhibitors of the conversion enzyme (Captopril, Enalapril), vasodilatators (Dihydralazine, Minoxidil, Prazozine,.) could provide a stimulation of the renin-angiotensin system.
   b. Beta adrenergic-blocking agents (Labetalol, Clonidin, Methyl-dopa,.) could provide inhibition on the renin-angiotensin system.
   c. Cathepsin B has been found to interfere with this test. Do not use this test for patients being administered Cathepsin B.

## 14. PERFORMANCE CHARACTERISTICS

### 14.1 Sensitivity

**Analytical Sensitivity (Limit of Detection)**
Mean signal (Zero-Calibrator) - 2SD

| | |
|---|---|
| Renin | 1 pg/mL |

**Functional Sensitivity**
Mean conc. < 20 % CV

| | |
|---|---|
| Renin | 5 pg/mL |

### 14.2 Measurement Range of the assay

1 - 320 pg/mL

### 14.3 Specificity

The specificity of the assay is guaranteed by the use of two complementary monoclonal antibodies. Human renin is recognized. No interference was observed when samples were spiked with any of the following substances : pro-renin, Cathepsin D (another enzyme of the aspartyl protease family), or various treatments for hypertension : Captopril, Renitec, Loxen, or Lasilix. Some interference was seen with Hemoglobin.
Note: Cathepsin B has been found to interfere with this test. Do not use this test for patients being administered Cathepsin B.

| Substance | [renin], pg/mL without substance | [renin], pg/mL with substance | Change in [renin] pg/mL | % Change |
|---|---|---|---|---|
| Lasilix, 50 µg/mL | 5.1 | 4.8 | 0.3 | 5.9 |
| | 10.7 | 10.1 | 0.6 | 5.6 |
| Cathepsin D, 0.5 U/mL | 5.1 | 5.6 | 0.5 | 9.8 |
| | 10.7 | 11.1 | 0.4 | 3.7 |
| Captopril, 50 µg / mL | 5.1 | 4.5 | 0.6 | 11.8 |
| | 10.7 | 10.5 | 0.2 | 1.9 |
| Renitec, 50 µg / mL | 5.1 | 4.9 | 0.2 | 3.9 |
| | 10.7 | 10.4 | 0.3 | 2.8 |
| Loxen, 50 µg/mL | 5.1 | 4.8 | 0.3 | 5.9 |
| | 10.7 | 10.8 | 0.1 | 0.9 |
| Cathepsin B, 0.1 U/mL | 5.1 | 9.4 | 4.3 | 84.0 |
| | 10.7 | 14.5 | 3.8 | 35.0 |

**14.4 Human Anti-Mouse Antibodies (HAMA)**

The immunoassay is protected against potential interferences with HAMA by adding a protection in the tracer of non-specific mouse immunoglobulins. Nevertheless, one cannot assure that there will never be a "false positive" result due to the presence of heterophilic antibodies in a patient sample.

**14.5 Pro-Renin**

A study was performed to evaluate the affect of Pro-Rennin on the Renin assay. The following results were obtained. It is determined that Cross Reaction with pro-renin is below 0.4 %.

| Samples | Renin measured pg/mL | Pro-renin measured pg/mL | % CR |
|---|---|---|---|
| Sample A | 24 | | |
| A + pro-renin (7.1ng/mL) | 46 | 22 | 0.31 |
| A + pro-renin (5.9ng/mL) | 42 | 18 | 0.30 |
| A + pro-renin (4.7ng/mL) | 37 | 13 | 0.27 |
| A + pro-renin (3.5ng/mL) | 35 | 10 | 0.30 |
| Sample B | 177 | | |
| B + pro-renin (8.3ng/mL) | 209 | 32 | 0.39 |
| B + pro-renin (7.1ng/mL) | 204 | 27 | 0.38 |
| B + pro-renin (5.9ng/mL) | 199 | 22 | 0.37 |
| B + pro-renin (4.7ng/mL) | 187 | 11 | 0.23 |
| B + pro-renin (3.5ng/mL) | 190 | 13 | 0.37 |

**14.6 Reproducibility**

An Intra-Assay study was performed to demonstrate the precision of the assay using seven (7) plasma samples that were measured 20 times in the same assay. The results are as follows:

Inter-Assay measurements were performed using six (6) plasma samples that were tested during a 3 year period ( more than 137 experiments ) during the release control process (including change of reagents, staff people etc. ). The following table summarizes the results of these assays during that time period.

The mean results of the measurements are shown in the tables below.

Determination of Intra-Assay variation

| Sample | Mean value ( pg / mL) | Calibrator deviation | CV ( % ) |
|---|---|---|---|
| A | 3.85 | 0.14 | 3.6 |
| B | 13.8 | 0.34 | 2.5 |
| C | 31.1 | 0.45 | 1.4 |
| G | 65.4 | 0.96 | 1.5 |
| E | 145 | 2.6 | 1.8 |
| H | 224 | 2.3 | 1.0 |
| F | 262 | 2.3 | 0.9 |

Determination of Inter-Assay variation

| | Plasma 1 | Plasma 2 | Plasma 3 | Plasma 4 | Plasma 5 | Plasma 6 |
|---|---|---|---|---|---|---|
| **N values** | 137 | 142 | 137 | 139 | 139 | 138 |
| **Mean (pg/mL)** | 4 | 14 | 32 | 72 | 146 | 263 |
| **SD (pg/mL)** | 0.2 | 0.6 | 1.2 | 2.6 | 5.4 | 10.5 |
| **C V (%)** | 5.0 | 4.3 | 3.7 | 3.6 | 3.7 | 4.0 |

**14.7 Recovery**

Increasing amounts of Renin were added to serum samples with various initial Renin concentrations. Each sample (non-spiked and spiked) was assayed in duplicates in one run. Renin concentrations were measured and the percentage recovery was calculated.

| Plasma No. | Endogenous Renin (pg/mL) | Added Renin (pg/mL) | Expected Renin (pg/mL) | Measured Renin (pg/mL) | Recovery (%) |
|---|---|---|---|---|---|
| 1 | 29.5 | 5 | 34.5 | 34.2 | 99 |
| 2 | 53.4 | 10 | 63.4 | 63.9 | 101 |
| 3 | 108 | 20 | 128 | 131 | 102 |

**14.8 Interference**

No interference with bilirubin, haemoglobin, and triglycerides, measured up to respective concentrations of equal to 250 mg/L, 1.25 g/L, and 20 g/L, has been observed.

The immunoassay is protected against any human anti-mouse antibody (HAMA) interference by the addition of a protector to the tracer (non-specific mouse immunoglobulins). However, we can not guarantee that this protection is exhaustive.

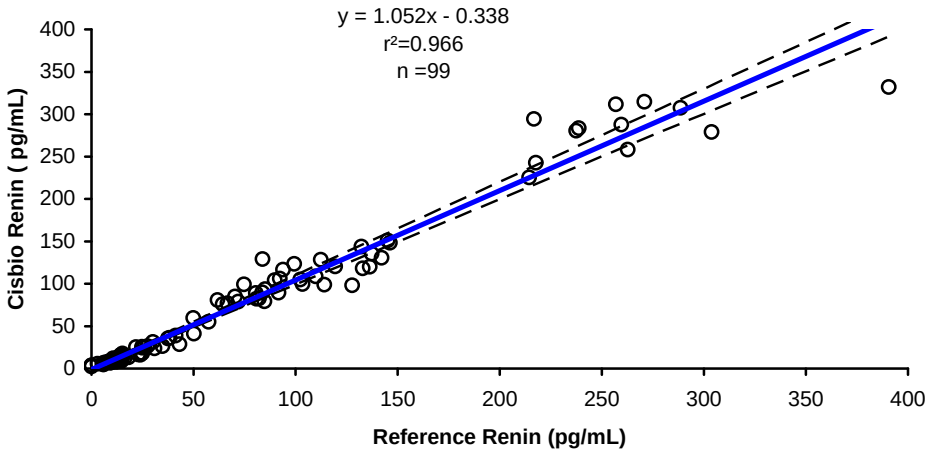
**14.9 Linearity**

Studies were performed to evaluate the linearity of the assay using EDTA plasma samples of different concentrations. The samples were assayed as neat and serially diluted with S0 down to 1:32 to demonstrate the full range of the assay. The following results were obtained.

| Dilution | Sample | Measured Conc. | Calculated Conc. x dilution factor | Recovery / Neat |
|---|---|---|---|---|
| NEAT | PL2 | 66.7 | | |
| 1/2 | | 30.2 | 60 | 91% |
| 1/4 | | 15.5 | 62 | 93% |
| 1/8 | | 7.9 | 63 | 94% |
| 1/16 | | 4.0 | 63 | 95% |
| 1/32 | | 2.0 | 65 | 97% |
| NEAT | PL5 | 41.0 | | |
| 1/2 | | 20.0 | 40 | 98% |
| 1/4 | | 10.6 | 42 | 103% |
| 1/8 | | 5.0 | 40 | 98% |
| 1/16 | | 2.6 | 41 | 101% |
| 1/32 | | 1.2 | 39 | 96% |
| NEAT | PL7 | 287.9 | | |
| 1/2 | | 141.3 | 283 | 98% |
| 1/4 | | 70.1 | 280 | 97% |
| 1/8 | | 35.3 | 282 | 98% |
| 1/16 | | 17.3 | 277 | 96% |
| 1/32 | | 8.8 | 281 | 97% |
| 1/64 | | 4.8 | 304 | 106% |
| 1/132 | | 2.3 | 298 | 104% |
| NEAT | B2 | 21.0 | | |
| 3/4 | | 14.6 | 19.5 | 93% |
| 1/2 | | 9.6 | 19.3 | 92% |
| 1/4 | | 5.0 | 19.9 | 95% |
| 1/8 | | 2.4 | 19.1 | 91% |
| 1/16 | | 1.2 | 19.8 | 94% |
| NEAT | C | 34.5 | | |
| 3/4 | | 24.7 | 32.9 | 95% |
| 1/2 | | 16.6 | 33.2 | 96% |
| 1/4 | | 8.3 | 33.3 | 97% |
| 1/8 | | 4.2 | 33.2 | 96% |
| 1/16 | | 2.1 | 33.4 | 97% |

## 15. METHOD COMPARISON

A study was performed to compare the results of the CISBIO Renin test to commercially available RIA test using patient plasma samples collected from a hospital laboratory. The samples were from 50 presumed normal male and female population and 49 patients identified as having hypertension (abnormal renin levels) or potentially having hypertension. The plasma samples were tested using the reference RIA Renin test and the CISBIO RIA Renin test. The results of the testing are shown in the Table below. The results of the testing yielded a regression formula of y = 1.052x – 0.338 with a correlation of 0.983 and $R^2$ = 0.966 for the ninety-nine patient samples evaluated throughout the dynamic range of the assay.




### ASSAY FLOW CHART

| Tubes | Calibrators (CAL 0 – CAL 5) Controls Samples µL | Tracer $^{125}$I µL | | Washing solution mL | | |
|---|---|---|---|---|---|---|
| **T** | - | 100 | Mix gently<br>----<br>Cover with Parafilm®<br>---<br>Incubate 3 h à 18-25°C under constant horizontal agitation<br>----<br>Aspirate. | - | Aspirate<br>---<br>Wash 2 times | Count for 2 minutes |
| **Calibrators** | 300 | 100 | | 2 | | |
| **Controls Samples** | 300 | 100 | | 2 | | |

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