Nori® Human Angiotensinogen ELISA Kit-DataSheet

Angiotensinogen (AGT) is a component of the renin-angiotensin system (RAS), a hormone system that regulates blood pressure and fluid balance.[1] It is also known as the renin substrate, and is a non-inhibitory member of the serpin family of proteinase inhibitors. Angiotensinogen is catalytically cleaved by renin to produce angiotensin I in response to lowered blood pressure. [2] Angiotensin converting enzyme (ACE), subsequently removes a dipeptide to produce angiotensin II, the physiologically active peptide, which functions in the regulation of volume and mineral balance of body. Angiotensin I and II can be further processed to generate angiotensin III, which stimulates aldosterone release and can be further processed by ACE to produce angiotensin 1-7, angiotensin 1-5 and angiotensin 1-4. Angiotensinogen is synthesized in the liver and secreted in plasma. Angiotensinogen appears to be associated with a predisposition to essential hypertension; it is also associated with pregnancy-induced hypertension (pih) (preeclampsia), a heterogeneous disorder that complicates 5-7% of all pregnancies and remains a leading cause of maternal, foetal and neonatal morbidity and mortality. Angiotensinogen plays a role in the process of Rheumatoid arthritis (RA) because angiotensin II is recognized to act as a powerful proinflammatory mediator.[3] It is also related to systemic lupus erythematosus and multiple sclerosis.

References

PRINCIPLE OF THE ASSAY

This is a quick ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance of the assays. An antibody specific for human angiotensinogen has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any angiotensinogen present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for human angiotensinogen is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution is added to the wells and color develops in proportion to the amount of angiotensinogen bound in the initial step. The color development is stopped and the intensity of the color is measured.

This package insert must be read in its entirety before using this product.

Storage
Store at 4°C and the kit can be used in 3 months.
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MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Description</th>
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<th>Description</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>Antibody Precoated Plate</td>
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<td>20 x PBS</td>
<td>1</td>
<td>Substrate Solution</td>
<td>1</td>
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<tr>
<td>Detection Antibody</td>
<td>1</td>
<td>20 x Wash Buffer</td>
<td>1</td>
<td>Stop Solution</td>
<td>1</td>
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<tr>
<td>Conjugate</td>
<td>1</td>
<td>10 x Reagent Diluent</td>
<td>1</td>
<td>DataSheet/Manual</td>
<td>1</td>
</tr>
<tr>
<td>Standard</td>
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<td>20 x Standard/Sample Diluent</td>
<td>1</td>
<td>96-well plate sheet</td>
<td>1</td>
</tr>
</tbody>
</table>

Bring all reagents to room temperature before use.

Reagent Preparations

**Human Angiotensinogen Detection Antibody** (1 vial) – The lyophilized Detection Antibody should be stored at 4°C for up to 3 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 200 µL of sterile 1 x PBS and vortex 30 sec. Take 200 µL of detection antibody to 10 mL of 1 x Reagent Diluent (*Working dilution of detection antibody*) if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

**Human Angiotensinogen Standard** (3 vials) – The lyophilized Human Angiotensinogen Standard has a total of 3 vials. Each vial contains the standard sufficient for a 96-well plate. The unreconstituted standard can be stored at 4°C for up to 3 months if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the tube. Add 500 µL of 1 x Standard/Sample Diluent to make the high standard concentration of 8000 pg/ml and vortex for 30 sec. A seven point standard curve is generated using 2-fold serial dilutions in the Standard/Sample Diluent, vortex 30 sec for each of dilution step.

**Conjugate** (50 µl) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 50 µL Conjugate sufficient for a 96-well plate. If the volume is less than 50 µL, add sterile 1 x PBS to reach 50 µL and vortex 10 sec. Make 1:200 dilutions in 1 x Reagent Diluent. If the entire 96-well plate is used, add 50 µL of Conjugate to 10 mL of 1 x Reagent Diluent to make *working dilution of Conjugate* prior to the assay. The rest of undiluted Conjugate can be stored at 4°C for up to 3 months. DO NOT FREEZE.

**20 x PBS, pH 7.3, 30 mL** - Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
**20 x Wash Buffer, 20 mL** - Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
**10 x Reagent Diluent** - Add 3 mL of sterile 1 x PBS to make 10 x Reagent Diluent, vortex 1 min and allow it to sit for 15 min to completely dissolve. Store at -20°C. Prior to use dilute to 1 x Reagent Diluent with 1 x PBS.
**20 x Standard/Sample Diluent**, 10 mL – Prior to use dilute to 1 x Sample Diluent with 1 x PBS and mix well.
**Substrate Solution**, 10 mL.
**Stop Solution**, 5 mL.
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**Assay Procedure**

1. Lift the plate cover from the top left and cover the wells that are not used. Vortex briefly the samples prior to the assay. Add 100 μL of sample (such as plasma or serum) or standard per well and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate 1 hour at room temperature.

2. Aspirate each well and wash with 1 x Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Wash Buffer (300 μL) using a multi-channel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

3. Add 100 μL of the working dilution of the Detection Antibody to each well. Cover the plate and incubate 1 hour at room temperature.

4. Repeat the aspiration/wash as in step 2.

5. Add 100 μL of the working dilution of Conjugate to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.

6. Repeat the aspiration/wash as in step 2.

7. Add 100 μL of Substrate Solution to each well. Incubate for 10-20 minutes at room temperature. Avoid placing the plate in direct light.

8. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well immediately, 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.

**Precaution and Technical Notes**

1. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected.

2. A standard curve should be generated for each set of samples assayed. Thorough mixing of standards at each of dilution steps is critical to acquire a normal standard curve.

3. If angiotensinogen exceeds the upper limit of the detection, the sample needs to be diluted with 1 x Standards/Sample Diluent. The dilution factor must be used for calculation of the concentration.

4. Conjugate contains enzyme, DO NOT mass up with Detection Antibody.

5. The Stop Solution is an acid solution, handle with caution.

6. This kit should not be used beyond the expiration date on the label.

7. A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by aspiration or by inverting the plate and blotting it against clean paper towels.

8. Use a fresh reagent reservoir and pipette tips for each step.

9. It is recommended that all standards and samples be assayed in duplicate.

10. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.
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**Calculation of Results**

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

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 angiotensinogen 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.

**The Standard Curve**

The graph below represents typical data generated when using this Human angiotensinogen ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark™ Microplate Reader and a Microplate Manager 6 Software were used to generate this curve. The correlation coefficient ($r^2$) is 0.999-1.000.
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Specificity
The following recombinant Human proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference. Adiponectin, ApoAI, BMP1, BMP2, BMP3, BMP4, BMP5, BMP7, CCL2, CCL4, CCL5, CRP, HSP27, HGF, IL-1 beta, IL-1RA, IL-2, IL-4, IL-5, IL-6, sIL-6R, IL-8, IL-10, IL-12, IL-15, IL-17C, IL-21, IL-23, IFNγ, MMP-2, MMP-9, IL2R, PDGF, serpin E1, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TLR9, TNF-α, TNF RI, TNF RII, VEGF, VEGF R1.

Calibration
This kit is calibrated against a highly purified CHO cell-expressed recombinant Human angiotensinogen.

Detection Range
125-8000 pg/ml

Assay Sensitivity
23 pg/ml

Assay Precision
Intra-Assay %CV: 7; Inter-Assay %CV: 10

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