Glucagon is a peptide hormone, produced by alpha cells of the pancreas. It works to raise the concentration of glucose in the bloodstream. Its effect is opposite that of insulin, which lowers the glucose.\cite{1} The pancreas releases glucagon when the concentration of glucose in the bloodstream falls too low. Glucagon causes the liver to convert stored glycogen into glucose, which is released into the bloodstream. High blood-glucose levels stimulate the release of insulin. Insulin allows glucose to be taken up and used by insulin-dependent tissues. Thus, glucagon and insulin are part of a feedback system that keeps blood glucose levels at a stable level. It increases energy expenditure and is elevated under conditions of stress.\cite{2} Glucagon belongs to a family of several other related hormones. Glucose is stored in the liver in the form of the polysaccharide glycogen, which is a glucan (a polymer made up of glucose molecules). Liver cells (hepatocytes) have glucagon receptors. When glucagon binds to the glucagon receptors, the liver cells convert the glycogen into individual glucose molecules and release them into the bloodstream, in a process known as glycogenolysis. As these stores become depleted, glucagon then encourages the liver and kidney to synthesize additional glucose by gluconeogenesis. Glucagon turns off glycolysis in the liver, causing glycolytic intermediates to be shuttled to gluconeogenesis. Glucagon also regulates the rate of glucose production through lipolysis. Glucagon induces lipolysis in humans under conditions of insulin suppression (such as diabetes mellitus type 1).\cite{4} Glucagon binds to the glucagon receptor, a G protein-coupled receptor, located in the plasma membrane. The conformation change in the receptor activates G proteins, a heterotrimeric protein with α, β, and γ subunits.

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 Glucagon has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Glucagon present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for human Glucagon 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 Glucagon 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 it can be used in 3 months.
Nori® Human Glucagon ELISA Kit-DataSheet

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Description</th>
<th>Quantity</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Precoated Plate</td>
<td>1</td>
<td>20 x PBS</td>
<td>1</td>
<td>Substrate Solution</td>
<td>1</td>
</tr>
<tr>
<td>Detection Antibody</td>
<td>1</td>
<td>20 x Assay 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>
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<tr>
<td>Standard</td>
<td>3</td>
<td>MSDS</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 Glucagon 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. If the entire 96-well plate is used, take 200 μL of detection antibody to 10.5 mL of 1 x Reagent Diluent to make **Working dilution of detection antibody** and mix thoroughly prior to the assay. If the partial antibody is used store the rest at -20°C until use.

**Human Glucagon Standard** (3 vials) – The lyophilized Human Glucagon Standard has a total of 3 vials. Each vial contains the standard sufficient for generating a standard curve. 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 Assay Buffer to make the high standard concentration of 2000 pg/ml and vortex for 30 sec. A seven-point standard curve is generated using 2-fold serial dilutions in the Assay Buffer, vortex 30 sec for each of dilution step.

**Conjugate** (53 μl) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 53 μL Conjugate sufficient for a 96-well plate. If the volume is less than 53 μL, add sterile 1 x PBS to reach 53 μL and vortex 10 sec. Make 1:200 dilutions in 1 x Reagent Diluent. If the entire 96-well plate is used, add 53 μL of Conjugate to 10.5 mL of 1 x Reagent Diluent to make **working dilution of Conjugate** and mix thoroughly 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 Assay Buffer, 20 mL– Dilute to 1 x Assay 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.
Substrate Solution, 10.5 mL.
Stop Solution, 5.5 mL.
Nori® Human Glucagon ELISA Kit-DataSheet

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 Assay Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Assay 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 Assay 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. Plasma or serum sample should be diluted with equal volume of 1 x Assay Buffer and vortex for 1 min prior to assay. If the OD value still exceeds the upper limit of the standard curve, further dilution is recommended till it falls in the detection range and 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.
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.
Nori® Human Glucagon ELISA Kit - DataSheet

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 Glucagon 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 Glucagon 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 (r²) is 0.999-1.000.

![Human Glucagon Standard Curve](image.png)
Nori® Human Glucagon ELISA Kit-DataSheet

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 Glucagon.

Detection Range
31-2000 pg/ml

Assay Sensitivity
6 pg/ml

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

For Research Use Only
## Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible causes</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>• Inaccurate pipetting&lt;br&gt; • Improper standard curve</td>
<td>• Check pipettes&lt;br&gt; • Check and use the correct dilution buffer&lt;br&gt; • Vortex 30 sec for each of standard dilution steps</td>
</tr>
<tr>
<td>Low signal</td>
<td>• Improper preparation of standard, samples, detection antibody, and/or conjugate&lt;br&gt; • Too brief incubation times&lt;br&gt; • Inadequate reagent volume or improper dilution</td>
<td>• Briefly spin down vials before opening. Reconstitute the powder thoroughly.&lt;br&gt; • Ensure sufficient incubation time.&lt;br&gt; • Check pipettes and ensure correct preparation.</td>
</tr>
<tr>
<td>Large CV</td>
<td>• Inaccurate pipetting and mixing&lt;br&gt; • Improper standard/sample dilutions.&lt;br&gt; • Air bubbles in wells.</td>
<td>• Check pipettes and ensure thorough mixing.&lt;br&gt; • Use the correct dilution buffers&lt;br&gt; • Remove bubbles in wells.</td>
</tr>
<tr>
<td>High background</td>
<td>• Plate is insufficiently washed.&lt;br&gt; • Contaminated Assay Buffer</td>
<td>• Review the datasheet for proper wash. If using a plate washer, ensure that all ports are unobstructed.&lt;br&gt; • Make fresh Assay Buffer</td>
</tr>
<tr>
<td>No signal detected</td>
<td>• The procedure was misconducted.</td>
<td>• Ensure the step-by-step protocol was correctly followed and no misstep was conducted.</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>• Improper storage of the ELISA kit&lt;br&gt; • Stop solution</td>
<td>• Store standards and detection antibody at -20°C after reconstitution, others at 4°C. Keep substrate protected from light.&lt;br&gt; • Adding stop solution to each well before reading plate</td>
</tr>
</tbody>
</table>