



## Nori® Chicken IgM ELISA MultiSet Kit DataSheet

Immunoglobulin M (IgM) is a basic antibody that is produced by B cells. IgM is by far the physically largest antibody in the human circulatory system. It is the first antibody to appear in response to initial exposure to an antigen. The spleen is the major site of specific IgM production. IgM forms polymers where multiple immunoglobulins are covalently linked together with disulfide bonds, mostly as a pentamer but also as a hexamer. The J chain is found in pentameric IgM but not in the hexameric form.<sup>[1]</sup> IgM has a molecular mass of approximately 970 kDa (in its pentamer form). Because each monomer has two antigen binding sites, a pentameric IgM has 10 binding sites. However, IgM cannot bind 10 antigens at the same time because the large size of most antigens hinders binding to nearby sites. Because IgM is a large molecule, it cannot diffuse well, and is found in the interstitium only in very low quantities. IgM is primarily found in serum; however, because of the J chain, it is also important as a secretory immunoglobulin. Due to its polymeric nature, IgM possesses high avidity, and is particularly effective at complement activation. By itself, IgM is an ineffective opsonin; however it contributes greatly to opsonization by activating complement and causing C3b to bind to the antigen.<sup>[2]</sup> IgM antibodies appear early in the course of an infection and usually reappear, to a lesser extent, after further exposure. IgM antibodies do not pass across the human placenta (only isotype IgG). These two biological properties of IgM make it useful in the diagnosis of infectious diseases. Demonstrating IgM antibodies in a patient's serum indicates recent infection, or in a neonate's serum indicates intrauterine infection (e.g. congenital rubella). The development of anti-donor IgM after organ transplantation is not associated with graft rejection but it may have a protective effect.<sup>[3]</sup>

### References

1. Erik J. et al, *J. Immunol.*, Jun 1998; 160: 5979 - 5989.
2. Wellek, B, et al. (1976). *Agents and Actions* 6 (1-3): 260-262.
3. Charpak, Y, et al. (2004). *Liver Transpl* 10 (2): 315-319.

### 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 Chicken IgM has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgM present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Chicken IgM 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 IgM 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. The kit can be used in 2 years.



## Nori® Chicken IgM ELISA MultiSet Kit DataSheet

### MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Capture Antibody	1	Detection Antibody	1	Standard	3
MSDS/CoA	1			DataSheet	1

Bring all reagents to room temperature before use.

### Reagent Preparations

This ELISA MultiSet kit contains sufficient materials for 10 of 96-well plates.

**Chicken IgM Capture Antibody** (1 vial) – Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. Refer to the lot-specific CoA for the amount supplied. Reconstitute the vial with 0.55 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the CoA. Store the vial at -20 °C after reconstitution.

**Biotinylated Chicken IgM Detection Antibody** (1 vial) – Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. Refer to the lot-specific CoA for the amount supplied. Reconstitute the vial with 0.55 mL of PBS. Dilute in PBS to the working concentration indicated on the CoA. Store the vial at -20 °C after reconstitution.

**Chicken IgM Standard** (3 vials) – Each of the lyophilized Chicken IgM Standard vial contains the standard sufficient for generating 15 standard curves. Refer to the lot-specific CoA for the amount supplied. Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. Reconstitute each vial with 200 µL of Assay Buffer. Prepare 500 µL of High Standard per plate assayed at the concentration indicated on the CoA with Assay Buffer. A seven-point standard curve is generated using 2-fold serial dilutions in the Assay Buffer, vortex 20 sec for each of dilution step. Store the vial at -20 °C after reconstitution.

### Other materials and solutions required but not supplied

1. **PBS**, pH 7.3, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, 0.2 µm filtered.
2. **Assay Buffer**, 0.05% Tween 20 in PBS, pH 7.3.
3. **Reagent Diluent**, 1% bovine serum albumin in PBS, pH 7.3.
4. **Streptavidin-Horseradish Peroxidase (SA-HRP) Conjugate**
5. **Substrate Solution**, mixture of H<sub>2</sub>O<sub>2</sub> and tetramethylbenzidine.
6. **Stop Solution**, 0.25 M H<sub>2</sub>SO<sub>4</sub>.
7. **96-well microplates with high binding.**



## Nori® Chicken IgM ELISA MultiSet Kit DataSheet

### Plate preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100  $\mu$ L per well of the diluted Capture Antibody and incubate for 2 hours at room temperature.
2. Aspirate each well and wash with 300  $\mu$ L of Assay Buffer per well and remove any remaining Assay Buffer by aspiration or by inverting the plate and blotting it against clean paper towel.
3. Block plates by adding 150  $\mu$ L of Reagent Diluent to each well. Incubate for 1 hour at room temperature.
4. Wash the plate as shown in Step 2.

### Assay Procedure

1. **Vortex the standards and samples for 10 sec** before applying to the plate. Add 100  $\mu$ L of **diluted sample** (see below) 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. Attention: **MUST vortex standards and samples for 10 sec before pipetting to the wells!**
2. Aspirate each well and wash with **1 x Assay Buffer**, repeating the process one time for a total of two washes. Wash by filling each well with 1 x Assay Buffer (300  $\mu$ L) 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  $\mu$ L of the **working dilution of 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  $\mu$ L of the **working dilution of SA-HRP 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  $\mu$ L of **Substrate Solution** to each well and observe the color development every 1-2 mins. Incubate for up to 20 minutes (**depending on signal. Stop** the reaction when it turns to dark blue in the highest standard wells) at room temperature. Over-incubation of the substrate will result in overflow of high standard and thus should be avoided. Avoid placing the plate in direct light.
8. When it gets to dark blue in the highest concentration of standard wells, add 50  $\mu$ L of **Stop Solution** to each well to stop the reaction. 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 and **vortex again (30 sec) before pipetting to the 96-well plate.**
3. HRP Conjugate contains enzyme, DO NOT mass up with Detection Antibody.
4. This kit should not be used beyond the expiration date on the label.



## Nori® Chicken IgM ELISA MultiSet Kit DataSheet

5. A thorough and consistent wash technique is essential for proper assay performance. Assay buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Assay buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
6. Use a fresh reagent reservoir and pipette tips for each step.
7. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

### 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 IGM 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 Chicken IgM 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.

### Specificity

The following recombinant Rat proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.

BMP4, IL-2, IL-4, IL-5, IL-6, IL-8, IFN $\gamma$ , TGF $\beta$ 1, TLR3, TNF- $\alpha$ .

### Calibration

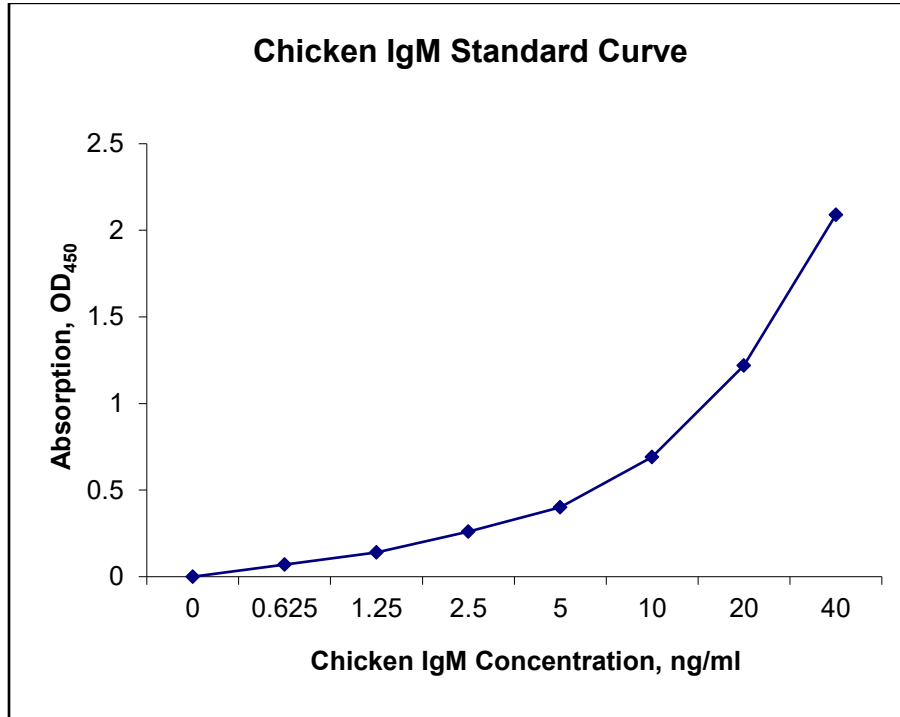
This kit is calibrated against chicken IgM.

**Detection Range**    0.6-40 ng/ml

**Assay Sensitivity**    100 pg/ml

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

## Nori® Chicken IgM ELISA MultiSet Kit DataSheet



### Related products

Chicken IgM Standard  
Chicken IgM detection antibody

### DECLARATION

THIS REAGENT IS FOR IN VITRO LABORATORY TESTING AND RESEARCH USE ONLY. DO NOT USE IT FOR CLINICAL DIAGNOSTICS. DO NOT USE OR INJECT IT IN HUMANS AND ANIMALS.

**FOR LABORATORY RESEARCH USE ONLY  
NOT FOR USE IN HUMANS AND ANIMALS**



## Nori® Chicken IgM ELISA MultiSet Kit DataSheet

### Troubleshooting Guide

Problem	Possible causes	Solution
Poor standard curve	<ul style="list-style-type: none"><li>• Inaccurate pipetting</li><li>• Insufficient vortexing</li><li>• OD<sub>450</sub> too high for the high standard point</li><li>• Air bubbles in wells.</li></ul>	<ul style="list-style-type: none"><li>• Check pipettes and ensure <b>equal dispensing</b>.</li><li>• Vortex 30 sec for each of standard dilution steps and <b>vortex again (10 sec) before pipetting to the 96-well plate</b>.</li><li>• Reduce substrate incubation time</li><li>• Remove air bubbles in wells by pipette tip.</li></ul>
Low signal	<ul style="list-style-type: none"><li>• Improper preparation of reagents and storage</li><li>• Too brief incubation times</li><li>• Inadequate reagent volume or improper dilution</li><li>• Standard defect</li></ul>	<ul style="list-style-type: none"><li>• Briefly spin down vials before opening. Reconstitute the powder thoroughly. Proper storage of plate and strip and detection antibody after first usage as shown in the datasheet.</li><li>• Ensure sufficient incubation time including substrate incubation. Increase sample incubation to 2 hours.</li><li>• <b>Change a Standard vial.</b></li></ul>
Overflow in the standards	<ul style="list-style-type: none"><li>• Substrate incubation too long</li><li>• Air bubbles in wells</li></ul>	<ul style="list-style-type: none"><li>• Observe the color development every 1-2 mins and reduce substrate incubation time.</li><li>• Remove air bubbles in wells</li><li>• Stop the reaction by adding 50 µl of Stop Solution when it turns to dark blue in the highest concentration of standard wells.</li></ul>
Large CV	<ul style="list-style-type: none"><li>• Inaccurate pipetting and mixing</li><li>• Improper standard/sample dilutions.</li><li>• Air bubbles in wells.</li></ul>	<ul style="list-style-type: none"><li>• Check pipettes and ensure accurate pipetting and thorough mixing and <b>equal dispensing</b>.</li><li>• Use the correct dilution buffers</li><li>• Remove air bubbles in wells by pipette tip.</li></ul>
High background	<ul style="list-style-type: none"><li>• Reagent reservoir issue</li><li>• Plate is insufficiently washed and air bubbles in wells.</li><li>• Contaminated wash buffer</li><li>• Pipet tip contaminated</li></ul>	<ul style="list-style-type: none"><li>• Use a new reagent reservoir for Substrate Solution.</li><li>• Increase wash to 4 times before adding substrate and ensure plate washer functions normally. Remove air bubbles in wells by pipette tip.</li><li>• Make fresh wash buffer and wash thoroughly.</li><li>• Use new pipette tips for blank wells.</li></ul>
No signal detected	<ul style="list-style-type: none"><li>• The procedure was misconducted.</li><li>• Failures of spin down the contents in Detection Antibody and Standards.</li><li>• Failure of Substrate or HRP</li><li>• Samples overdiluted</li></ul>	<ul style="list-style-type: none"><li>• Ensure the step-by-step protocol. Spin vials of Detection antibody and Standard to complete recover the content.</li><li>• Mix 100 µl of Substrate with 0.5 µl HRP and deep blue color should develop in 2 min.</li><li>• Try a new standard vial and use positive control.</li><li>• Try not dilute samples</li></ul>
Low sensitivity	<ul style="list-style-type: none"><li>• Improper dilutions of standards</li><li>• Improper storage of the ELISA kit</li></ul>	<ul style="list-style-type: none"><li>• Ensure accurate and thorough dilutions of standards at each step.</li><li>• Store detection antibody at -20°C after reconstitution, others at 4°C. Keep substrate solution protected from light.</li></ul>