Nori® Mouse TNFRII ELISA Kit DataSheet

Tumor necrosis factor receptor 2 (TNFR2), also known as tumor necrosis factor receptor superfamily member 1B (TNFRSF1B) and CD120b, is a membrane receptor that binds tumor necrosis factor-alpha (TNFα).[1] The protein is a member of the tumor necrosis factor receptor superfamily, which also contains TNFRSF1A. This protein and TNF-receptor 1 form a heterocomplex that mediates the recruitment of two anti-apoptotic proteins, c-IAP1 and c-IAP2, which possess E3 ubiquitin ligase activity. The function of IAPs in TNF-receptor signaling is unknown, however, c-IAP1 is thought to potentiate TNF-induced apoptosis by the ubiquitination and degradation of TNF-receptor-associated factor 2 (TRAF2), which mediates anti-apoptotic signals. Knockout studies in mice also suggest a role of this protein in protecting neurons from apoptosis by stimulating antioxidative pathways. TNFRSF1B has been shown to interact with TRAF2,[2] and TTRAP.[3] "A gene mutation of TNFR2 is associated with systemic lupus erythematosus: a case-control study and a meta-analysis.[4] TNFRII interacts with members of the TNFR-associated factor family and activates the transcription factors NF-kappaB and AP-1.[5]

References

1. Schall TJ, et al. (1990). Cell. 61 (2): 361–70.
2. Carpentier I, et al. (2008). Biochem. Biophys. Res. Commun. 374 (4): 752–7.
3. Pype S, (2000). J. Biol. Chem. 275 (24): 18586–93.
4. Horiuchi T, et al. (2007). Ann. Rheum. Dis. 66 (3): 320–4.
5. Marsters SA, et al. (1997). J. Biol. Chem. 272 (22): 14029–32.

PRINCIPLE OF THE ASSAY

This kit is for quantification of total TNFRII. This is a shorter 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 and the sensitivity of the assays. An antibody specific for Mouse TNFRII has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNFRII present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Mouse TNFRII 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 TNFRII 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 6 months.

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Nori® Mouse TNFRII ELISA Kit DataSheet

MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	20 x Assay Buffer	1	Stop Solution	1
HRP Conjugate	1	96-well plate sheet	1	DataSheet/Manual	1
Standard	3				

Bring all reagents to room temperature before use.

Reagent Preparatins

Mouse TNFRII Detection Antibody (1 vial) – The lyophilized Detection Antibody should be stored at 4°C or -20°C in a manual defrost freezer for up to 6 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 to the antibody vial and vortex for 20 sec and allow it to sit for 5 min prior to open the vial. If the entire 96-well plate is used, take 200 µL of detection antibody to 10.5 mL of 1 x PBS 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.

Mouse TNFRII Standard (3 vials) – Each lyophilized Mouse TNFRII Standard vial contains the standard sufficient for generating a standard curve. The non-reconstituted standard can be stored at 4°C or -20°C for up to 6 months if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. Add 500 µL of 1 x Assay Buffer to one Standard vial to make the high standard concentration of 600 pg/ml. Vortex for 20 sec and allow it to sit for 5 min prior to use. A seven-point standard curve is generated using 2-fold serial dilutions in 1 x Assay Buffer, each in duplicate, vortex for 20 sec for each of dilution steps.

HRP Conjugate (55 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient HRP Conjugate for one 96-well plate. If the volume is less than 55 µL, add sterile 1 x PBS to a final volume of 55 µL and vortex briefly. Make 1:200 dilutions in 1 x PBS. If the entire 96-well plate is used, add 53 µL of HRP Conjugate to 10.5 mL of 1 x PBS prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months. DO NOT FREEZE.

20 x PBS, pH 7.3, 25 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.
Substrate Solution, 10.5 mL.
Stop Solution, 5.5 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 **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.
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 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 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 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 µL of **Substrate Solution** to each well. Incubate for 5-20 minutes (**depending on signal**) at room temperature. Avoid placing the plate in direct light. **Ensure all wells turn yellow by pipette tip prior to measurement.**
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.

Sample dilution: Samples should be diluted with four volumes 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.

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 (10 sec) before pipetting to the 96-well plate**.
3. HRP Conjugate contains enzyme, DO NOT mass up with Detection Antibody.
4. The Stop Solution is an acid solution, handle with caution.
5. This kit should not be used beyond the expiration date on the label.
6. A thorough and consistent wash technique is essential for proper assay performance.
7. Use a fresh reagent reservoir and pipette tips for each step.
8. It is recommended that all standards and samples be assayed in duplicate.
9. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

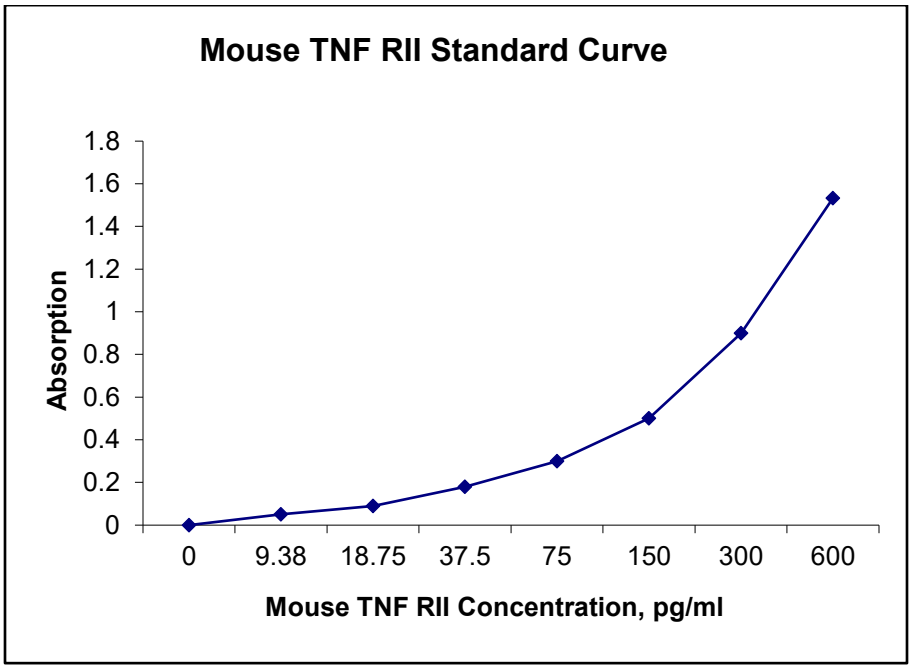
Calculation of Results

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 TNFRII 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 Mouse TNFRII ELISA Kit. The correlation coefficient (r^2) is 0.995-1.000. The standard curve should be calculated using a computer generated 4-PL curve-fit to determine concentrations of unknow specimens.



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Nori® Mouse TNFRII ELISA Kit DataSheet

Specificity

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

ApoAI, BMP4, HGF, HSP27, IFNγ, IL-1β, IL-1RA, MMP-2, MMP-9, sIL-2R, sIL-6R, TGFβ1, TLR3, TNF-α, TNF RI, VEGF.

Detection Range

9.38-600 pg/ml

Assay Sensitivity

2 pg/ml

Assay Precision

Intra-Assay %CV: 4; Inter-Assay %CV: 9

Sample types: plasma, serum, cell/tissue lysates, cell culture supernatant, synovial fluid (SF), bronchoalveolar lavage (BALF), cerebrospinal fluid (CSF), urine, other biological fluid.

Related products

1. GR238016 50 ml Reagent Reservoir, 100/case, 5 packs/case (pack of 20)
2. GR238004 Tissue Culture 96-well Microplate, individually packed, Case of 50
3. GR238019 1.5 ml Microcentrifuge tube with screw cap and free-standing, pack of 500
4. GR238007 125 ml leak-resistant HDPE bottle, colorless, pack of 24
5. GR238002 Microplate 12x8-Well Strip High Binding, Case of 50
6. GR238003 Microplate 12x8-Well Strip Medium Binding, Case of 50
7. GR238032 42592 Costar Stripwell Microplate 1 x 8 Flat Bottom, High Binding, Case of 100
8. GR238001 468667 Thermo Microplate 12x8-Well Strip Nunc Maxixorp F8, Case of 60
9. GR238031 96-well microplate sealer plastic, pack of 100

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

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Troubleshooting Guide

Problem	Possible causes	Solution
Poor standard curve	• Inaccurate pipetting • Insufficient vortexing • OD_{450} too high for the high standard point • Air bubbles in wells. • Standard defect or not fully recovered	• Check pipette calibration and ensure equal dispensing. • Vortex 30 sec for each of standard dilution steps and vortex again (10 sec) before pipetting to the 96-well plate. • Reduce substrate incubation time • Remove air bubbles in wells by pipette tip. • Change a standard vial or spin down the vial before reconstitution
Low signal	• Improper preparation of reagents and storage • Too brief incubation times • Inadequate reagent volume or improper dilution • Standard defect and sample overdiluted	• Briefly spin down vials before opening. Reconstitute the content thoroughly. Proper storage of plate and strip and detection antibody after first usage as shown in the datasheet. Microplate shaker may improve signals. • Ensure sufficient incubation time including substrate incubation. Increase sample incubation to 2 hours. • Change a Standard vial. Undilute sample or less dilution
Overflow in the standards	• Substrate incubation too long • Air bubbles in wells	• Observe the color development every 1-2 mins and reduce substrate incubation time. • Stop the reaction by adding 50 µl of Stop Solution when it turns to dark blue in the highest concentration of standard wells. • Remove air bubbles in wells
Large CV	• Inaccurate pipetting and mixing • Improper standard/sample dilutions. • Air bubbles in wells. • Microplate reader out of calibration • It did not turn yellow after adding Stop Solution	• Check pipettes and ensure the pipette is calibrated properly. • Ensure accurate pipetting and thorough mixing. • Use reverse, instead of forward pipetting. • Use the correct dilution buffers • Remove air bubbles in wells by pipette tip. • Calibrate the microplate reader properly • If it did not turn yellow after adding Stop Solution, mix with pipette tip till it turns yellow prior to measurement.
High background	• Reagent reservoir issue • Plate is insufficiently washed and air bubbles in wells. • Contaminated Assay Buffer • Pipet tip contaminated	• Use a new reagent reservoir for Substrate Solution. • Increase wash to 4 times before adding substrate and ensure plate washer functions normally. Remove air bubbles in wells by pipette tip. Use squirt bottle for washing. • Make fresh Assay Buffer and wash thoroughly. • Use new pipette tips for blank wells.
No signal detected	• The procedure was misconducted. • Failures of spin down the contents in Detection Antibody and Standards. • Failure of Substrate or HRP • Samples overdiluted	• Ensure the step-by-step protocol. Spin vials of Detection antibody and Standard to completely recover the content. • Mix 100 µl of Substrate with 0.5 µl HRP and dark blue color should develop in 5 min. • Try a new standard vial and use positive control. • Try not dilute samples
Low sensitivity	• Improper dilutions of standards • Improper storage of the ELISA kit	• Ensure accurate and thorough dilutions of standards at each step. • Store detection antibody at -20°C after reconstitution and others at 4°C. Keep substrate solution protected from light.