Nori® Rat CXCR4 ELISA Kit-DataSheet

C-X-C chemokine receptor type 4 (CXCR-4) also known as fusin or CD184 is a protein that is encoded by the CXCR4 gene.[1] CXCR-4 is an alpha-chemokine receptor specific for stromal-derived-factor-1 (SDF-1 also called CXCL12), a molecule endowed with potent chemotactic activity for lymphocytes.[2] HIV uses CXCR4 to infect CD4+ T cells. It is unclear as to whether the emergence of CXCR4-using HIV is a consequence or a cause of immunodeficiency. CXCR4 is upregulated during the implantation window in natural and hormone replacement therapy cycles in the endometrium, producing, in presence of a Rat blastocyst, a surface polarization of the CXCR4 receptors suggesting that this receptor is implicated in the adhesion phase of Rat implantation. CXCR4's ligand SDF-1 is known to be important in hematopoietic stem cell homing to the bone marrow and in hematopoietic stem cell quiescence. It has been also shown that CXCR4 signaling regulates the expression of CD20 on B cells. Until recently, SDF-1 and CXCR4 were believed to be a relatively monogamous ligand-receptor pair. Recent evidence demonstrates ubiquitin is also a natural ligand of CXCR4.[3] Ubiquitin is best known for its intracellular role in targeting ubiquitylated proteins for degradation via the ubiquitin proteasome system. Evidence in numerous animal models suggests ubiquitin is anti-inflammatory immune modulator and endogenous opponent of proinflammatory damage associated molecular pattern molecules. MIF is an additional ligand of CXCR4.[4] CXCR4 is present in newly generated neurons during embryogenesis and adult life where it plays a role in neuronal guidance. The levels of the receptor decrease as neurons mature. CXCR4 mutant mice have aberrant neuronal distribution. This has been implicated in disorders such as epilepsy.[5] CXCR4 dimerization is dynamic and increases with concentration.[6]

References

1. Moriuchi M, et al. (1997). Journal of Immunology. 159 (9): 4322–9.
2. Kryczek I, et al. (2007). American J Physiol. Cell Physiol. 292 (3): C987-95.
3. Saini V, et al. (2010). The Journal of Biological Chemistry. 285 (20): 15566–76.
4. Bernhagen J, et al. (2007). Nature Medicine. 13 (5): 587–96.
5. Bagri A, et al. (2002). Development. 129 (18): 4249–60.
6. Işbilir A, et al. (2020). Proc Natl Acad Sci USA. 117 (46): 29144–29154.

PRINCIPLE OF THE ASSAY

This ELISA kit is for quantification of CXCR4 in Rat samples. 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 Rat CXCR4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CXCR4 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Rat CXCR4 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 CXCR4 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 the kit at 4°C. The kit can be used in 6 months.

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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	1
Standard	3				

Bring all reagents to room temperature before use.

Reagent Preparations

Rat CXCR4 Detection Antibody (1 vial) – The lyophilized Detection Antibody should be stored at 4°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. 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 PBS to make **Working dilution of detection antibody** and vortex 30 sec prior to the assay. If the partial antibody is used store the rest at -20°C until use.

Rat CXCR4 Standard (3 vials) – Each lyophilized Rat CXCR4 Standard vial contains the standard sufficient for generating a standard curve. The unreconstituted standard can be stored at 4°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 tube. Add 500 µL of 1 x Assay Buffer to make the high standard concentration of 8 ng/ml and **vortex for 1 min**. A seven-point standard curve is generated using 2-fold serial dilutions in the Assay Buffer, **vortex 30 sec** for each of dilution step.

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 55 µL HRP Conjugate sufficient for one 96-well plate. If the volume is less than 55 µL, add sterile 1 x PBS to reach 55 µL and vortex 10 sec. 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 to make **working dilution of HRP Conjugate** and vortex 30 sec 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.

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

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Assay Procedure

1. All procedures are conducted at room temperature (20-25 ºC) and ensure **equal pipetting/dispensing** at each step and remove air bubbles in the wells for all steps.
2. Lift the plate cover and cover the unused wells or reseal the unused strips in the aluminum bag with desiccant at 4 ºC. **Vortex the standards and samples for 10 sec** before applying to the plate. Add 100 µL of **sample** or **standard** per well and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate for **2 hours**. Attention: **MUST vortex standards and samples for 10 sec before pipetting to the wells!**
3. Aspirate each well and wash with 300 µL of **1 x Assay Buffer** for two times. Wash by filling each well with 1 x Assay Buffer 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.
4. Add 100 µL of the **working dilution of Detection Antibody** to each well. Cover the plate and incubate for 1 hour.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µL of the **working dilution of HRP Conjugate** to each well. Cover the plate and incubate for 20 minutes. Avoid placing the plate in direct light.
7. Repeat the aspiration/wash as in step 3 but **wash 4 times** instead.
8. Add 100 µL of **Substrate Solution** to each well and observe the color development every 1-2 mins. Incubate for up to **30 minutes** (**depending on signal. Stop** the reaction when it turns to dark blue in the highest standard wells). Over-incubation of the substrate will result in overflow of high standard and thus should be avoided. Avoid placing the plate in direct light.
9. When it gets to dark blue in the highest concentration of standard wells, add 50 µL of **Stop Solution** to each well to stop the reaction. Gently tap the plate to ensure thorough mixing.
10. 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: If high density is expected, samples 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.

Precaution and Technical Notes

1. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected and make sure no air bubbles in wells before adding reagents.
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 performance of the assay.

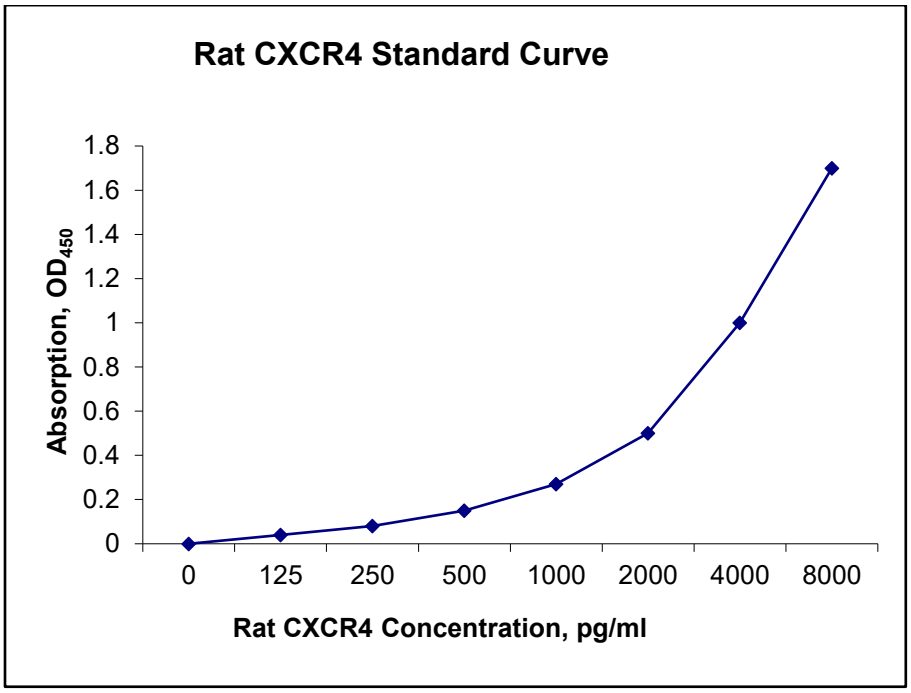
Calculation of Results

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 CXCR4 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 Rat CXCR4 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 Rat proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
ApoAI, BMP1, CRP, HGF, HSP27, IL-1α, IFNγ, MMP-2, PDGF, PLA2G7, prolactin, TGFβ1, TLR3, TNF-α, VEGF.
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Calibration

This kit is calibrated against a highly purified recombinant rat CXCR4.

Detection Range

125-8,000 pg/ml

Assay Sensitivity

25 pg/ml

Assay Precision

Intra-Assay %CV: 6; Inter-Assay %CV: 9

Related products

Rat CXCR4 Standard
Rat CXCR4 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 RATS AND ANIMALS

Troubleshooting Guide

Problem	Possible causes	Solution
Poor standard curve	• Inaccurate pipetting • Improper standard curve • OD_{450} too high for the high standard point	• Check pipettes • Check and use the correct dilution buffer • 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
Low signal	• Improper preparation of standard, samples, detection antibody, and/or HRP Conjugate • Too brief incubation times • Inadequate reagent volume or improper dilution	• Briefly spin down vials before opening. Reconstitute the powder thoroughly. • Ensure sufficient incubation time including substrate incubation. • Check pipettes and ensure correct preparation.
Overflow in the standards	• Substrate incubation too long	• Reduce substrate incubation time. • Observe the color development every 1-2 mins. • Stop the reaction by adding 50 µl of Stop Solution when it turns to dark blue in the highest concentration of standard wells.
Large CV	• Inaccurate pipetting and mixing • Improper standard/sample dilutions. • Air bubbles in wells.	• Check pipettes and ensure the pipette is calibrated. • Ensure thorough mixing and equal dispensing. • Use the correct dilution buffers • Remove bubbles in wells.
High background	• Plate is insufficiently washed. • Contaminated wash buffer	• Review the datasheet for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
No signal detected	• The procedure was misconducted.	• Ensure the step-by-step protocol was correctly followed and no misstep was conducted.
Low sensitivity	• Improper storage of the ELISA kit • Stop solution	• Store standards and detection antibody at -20°C after reconstitution, others at 4°C. Keep substrate protected from light. • Adding stop solution to each well before reading plate