# PRODUCT INFORMATION & ELISA MANUAL

# CXCL2/GRO beta/MIP-2/CINC-3 Antibody Pair [HRP]
## *NBP2-79491*
## Sample Insert for reference use only

Matched Antibody Pair utilized in an Enzyme-linked Immunosorbent Assay for quantitative detection of Mouse CXCL2/GRO beta/MIP-2/CINC-3.

For research use only.

Not for diagnostic or therapeutic procedures.

## BACKGROUND

Chemokine (C-X-C motif) ligand 2 (CXCL2), also called macrophage inflammatory protein 2 (MIP-2), Growth-regulated protein beta (Gro-beta) and Gro oncogene-2 (Gro-2), is a small cytokine belonging to the CXC chemokine family. CXCL2/MIP-2 is selectively up-regulated in tolerance-conferring APCs and serves to recruit NKT cells to the splenic marginal zone, where they form clusters with APCs and T cells. In the absence of the high-affinity receptor for CXCL2/MIP-2 or in the presence of a blocking Ab to CXCL2/MIP-2, peripheral tolerance is prevented, and Ag-specific T regulatory cells are not generated. CXCL2/MIP-2 is selectively up-regulated in tolerance-conferring APCs and serves to recruit NKT cells to the splenic marginal zone, where they form clusters with APCs and T cells. In the absence of the high-affinity receptor for MIP-2 (as in CXCR2-deficient mice) or in the presence of a blocking Ab to MIP-2, peripheral tolerance is prevented, and Ag-specific T regulatory cells are not generated. Understanding the regulation of lymphocyte traffic during tolerance induction may lead to novel therapies for autoimmunity, graft acceptance, and tumor rejection. Several studies have implicated the CXCL2 chemokine as a mediator in the development of sepsis. CXCL2/MIP-2 also plays a major role in mediating the neutrophilic inflammatory response of the rodent lung to particles such as quartz, crocidolite asbestos, as well as high doses of other relative innocuous dusts such as titanium dioxide.

## PRINCIPLE OF THE TEST

The Novus Biologicals CXCL2/GRO beta/MIP-2/CINC-3 Antibody Pair [HRP] is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for Mouse CXCL2/GRO beta/MIP-2/CINC-3 coated on a 96-well plate. Standards and samples are added to the wells, and any Mouse CXCL2/GRO beta/MIP-2/CINC-3 present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated rabbit anti-Mouse CXCL2/GRO beta/MIP-2/CINC-3 polyclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of Mouse CXCL2/GRO beta/MIP-2/CINC-3 present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

## INTENDED USE

◆ The Mouse CXCL2/GRO beta/MIP-2/CINC-3 Antibody Pair [HRP] is for the quantitative determination of Mouse CXCL2/GRO beta/MIP-2/CINC-3.

◆ This CXCL2/GRO beta/MIP-2/CINC-3 Antibody Pair [HRP] contains the basic components required for the development of sandwich ELISAs.

## ASSAY PROCEDURE SUMMARY
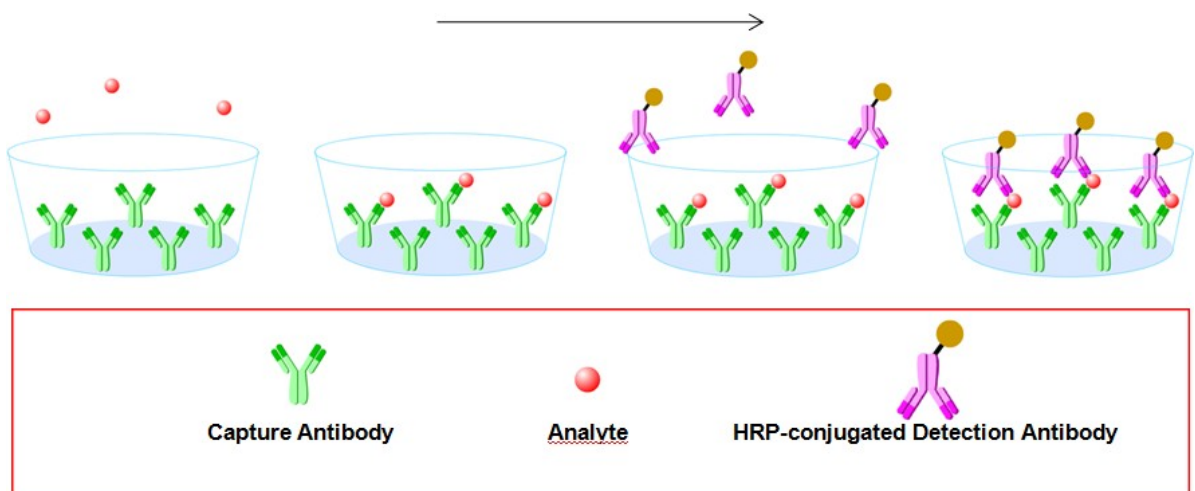


**This antibody pair has been configured for research use only and is not to be used in diagnostic procedures.**

# MATERIALS PROVIDED

**Bring all reagents to room temperature before use.**

**Capture Antibody** – 1 mg/mL of rabbit anti-Mouse CXCL2/GRO beta/MIP-2/CINC-3 monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 2 µg/mL in PBS before coating.

**Detection Antibody** – 0.2 mg/mL of rabbit anti-Mouse CXCL2/GRO beta/MIP-2/CINC-3 polyclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4, store at 4℃). Dilute to working concentration of 0.5 µg/mL in detection antibody dilution buffer before use.

**Standard** – Each vial contains 7.3 ng of recombinant Mouse CXCL2/GRO beta/MIP-2/CINC-3. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20℃ to -80℃ in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 250 pg/mL is recommended.

# SOLUTIONS REQUIRED

**PBS** - 136.9 mM NaCl, 10.1 mM $Na_2HPO_4$, 2.7 mM KCl, 1.8 mM $KH_2PO_4$, pH 7.4, 0.2 µm filtered

**Wash Buffer** - 0.05% Tween20 in PBS, pH 7.2 - 7.4

**Blocking Buffer** - 2% BSA in Wash Buffer

**Sample dilution buffer** - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

**Detection antibody dilution buffer** - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

**Substrate Solution :** To achieve best assay results, fresh substrate solution is recommended

**Substrate stock solution** - 10mg / ml TMB ( Tetramethylbenzidine ) in DMSO

**Substrate dilution buffer** - 0.05M $Na_2HPO_4$ and 0.025M citric acid ; adjust pH to 5.5

**Substrate working solution** - For each plate dilute 250 µl substrate stock solution in 25ml substrate dilution buffer and then add 80 µl 0.75% $H_2O_2$ , mix it well

**Stop Solution** - 2 N $H_2SO_4$

# PRECAUTION

The Stop Solution suggested for use with this antibody pair is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

## STORAGE

**Capture Antibody**: Aliquot and store at -20℃ to -80℃ for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

**Detection Antibody**: Store at 4℃ and protect it from prolonged exposure to light for up to 6 months from date of receipt. **DO NOT FREEZE!**

**Standard**: Store lyophilized standard at -20℃ to -80℃ for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -80℃ for up to 1 month. Avoid repeated freeze-thaw cycles.

# GENERAL ELISA PROTOCOL

## Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS. Immediately coat a 96-well microplate with 100µL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4℃.
2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

## Assay Procedure

1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 µL of substrate solution to each well. Incubate for 20 minutes at room temperature ( **if substrate solution is not as requested, the incubation time should be optimized** ). Avoid placing the plate in direct light.
6. Add 50 µL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

# CALCULATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
- 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.
- To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

## TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.
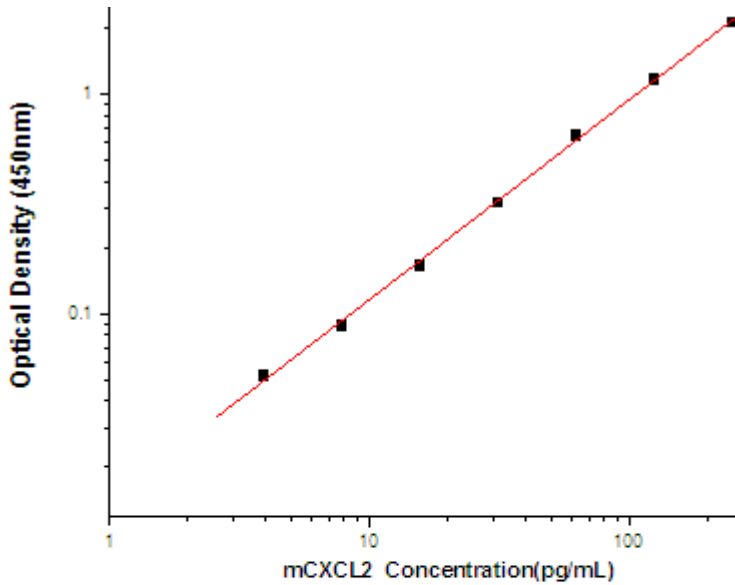


| Concentration ( pg/mL) | Zero standard subtracted OD |
|---|---|
| 0 | 0 |
| 3.91 | 0.052 |
| 7.82 | 0.088 |
| 15.63 | 0.166 |
| 31 | 0.322 |
| 62.5 | 0.651 |
| 125 | 1.175 |
| 250 | 2.122 |

## PERFORMANCE CHARACTERISTIC

## SENSITIVITY

The minimum detectable dose of Mouse CXCL2/GRO beta/MIP-2/CINC-3 was determined to be approximately **3.91 pg/ml**. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

# TROUBLE SHOOTING

| Problems | Possible Sources | Solutions |
|---|---|---|
| **No signal** | Incorrect or no Detection Antibody was added | Add appropriate Detection Antibody and continue |
| | Substrate solution was not added | Add substrate solution and continue |
| | Incorrect storage condition | Check if the kit is stored at recommended condition and used before expiration date |
| **Poor Standard Curve** | Standard was incompletely reconstituted or was inappropriately stored | Aliquot reconstituted standard and store at -80 ℃ |
| | Imprecise / inaccurate pipetting | Check / calibrate pipettes |
| | Incubations done at inappropriate temperature, timing or agitation | Follow the general ELISA protocol |
| | Background wells were contaminated | Avoid cross contamination by using the sealer appropriately |
| **Poor detection value** | The concentration of antigen in samples was too low | Enriching samples to increase the concentration of antigen |
| | Samples were ineffective | Check if the samples are stored at cold environment. Detect samples in timely manner |
| **High Background** | Insufficient washes | Use multichannel pipettes without touching the reagents on the plate |
| | | Increase cycles of washes and soaking time between washes |
| | TMB Substrate Solution was contaminated | TMB Substrate Solution should be clear and colorless prior to addition to wells |
| | Materials were contaminated. | Use clean plates, tubes and pipettes tips |
| **Non-specificity** | Samples were contaminated | Avoid cross contamination of samples |
| | The concentration of samples was too high | Try higher dilution rate of samples |

## ELISA Plate Template

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | | | | | | | | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

# Mouse CXCL2/GRO beta/MIP-2/CINC-3 Antibody Pair [HRP]

# Notes