Monoclonal Anti-rat CINC-2α/β Antibody

ORDERING INFORMATION

Catalog Number: MAB516

Clone: 83504

Lot Number: FZO02

Size: 500 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

Specificity: rat CINC-2α and CINC-2β

Immunogen: *E. coli*-derived rrCINC-2α and rrCINC-2β

Ig class: mouse IgG_{2B}

Applications: Neutralization of bioactivity
Western blot
ELISA capture

Figure 1

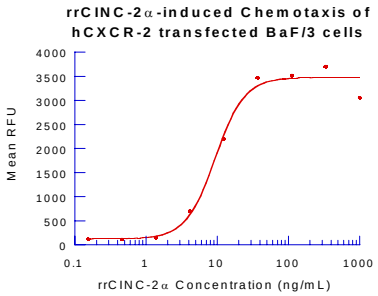
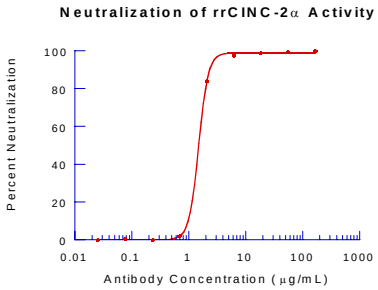


Figure 2



Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with a cocktail of purified, *E. coli*-derived, recombinant rat cytokine-induced neutrophil chemoattractant 2 alpha and 2 beta (rrCINC-2α and rrCINC-2β). The IgG fraction of the ascites fluid was purified by Protein G affinity chromatography. CINC-2α and -2β are mRNA splice variant isoforms that differ by only 3 amino acids at their amino-termini. These proteins are CXC chemokines most closely related to human GROs and mouse MIP-2. They are produced by granulation tissue, activated macrophages and fibroblasts. CINC-2 biological activities are mediated by CXCR1 or CXCR2.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Endotoxin Level

< 0.1 EU per 1 µg of the antibody as determined by the LAL method.

Reconstitution

Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 500 µg/mL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody was selected for its ability to neutralize the biological activity of rat CINC-2α and rat CINC-2β and for use as a capture antibody in rat CINC-2α/β sandwich ELISAs.

Applications

Neutralization of Rat CINC-2α Bioactivity - The exact concentration of antibody required to neutralize rat CINC-2α activity is dependent on the cytokine concentration, cell type, growth conditions and the type of activity studied. To provide a guideline, R&D Systems has determined the neutralization dose for this antibody under a specific set of conditions. The **Neutralization $Dose_{50}$ (ND_{50})** for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.

The ND_{50} for this lot of anti-rat CINC-2α/β antibody was determined to be approximately 0.5 - 2.5 µg/mL in the presence of 0.05 µg/mL of rrCINC-2α and 1 - 4 µg/mL in the presence of 0.05 µg/mL of rrCINC-2β. The specific conditions are described in the figure legends.

Figure 1: Rat CINC-2α can induce the chemotaxis of hCXCR-2 transfected BaF/3 cells in a dose-dependent manner. The ED_{50} for this effect is typically 4 - 20 ng/mL.

Figure 2: Approximately 0.5 - 2.5 µg/mL of the antibody will neutralize 50% of the bioactivity due to 0.05 µg/mL of rrCINC-2α.

Western Blot - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect rat CINC-2α or CINC-2β. The detection limit for rrCINC-2α is approximately 5 ng/lane under non-reducing and reducing conditions. In western blots, this antibody shows no cross-reactivity with other chemokines tested.[1]

ELISA Capture - This antibody can be used as a capture antibody in a rat CINC-2α/β ELISA in combination with biotinylated, rat CINC-2α/β affinity purified polyclonal detection antibody (Catalog # BAF516). A general protocol is provided on the next page. Using plates coated with 100 µL/well of the capture antibody at 2 µg/mL, in combination with 100 µL/well of the detection antibody, an ELISA for sample volumes of 100 µL can be obtained. To arrive at the optimal dose range for this ELISA, set up a two-fold dilution series of the protein standard starting with 2 ng/mL. Cross-reactivities observed in this format were 27.9% with rrCINC-3 and no cross-reactivity or interference was observed with rrCINC-1, rmKC, rmMIP-2 or rhGROα.

Optimal dilutions should be determined by each laboratory for each application.

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ELISA Protocol

Solutions Required

- **Wash Buffer** - 0.05% Tween® 20 in PBS, pH 7.4
- **Diluent** - 1% BSA in Phosphate-buffered Saline pH 7.4.
- **Substrate Solution** - 1:1 mixture of Color Reagent A (H_20_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999)
- **Stop Solution** - 1 M H_2SO_4

Plate Preparation

1. Transfer 100 µL/well of the capture antibody (diluted to the appropriate concentration in PBS, use immediately) to an ELISA plate. Seal plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash buffer, repeating the process two times for a total of 3 washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper toweling.
3. Block plates by adding 300 µL of PBS containing 1% BSA, 5% sucrose and 0.05% NaN_3 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. Alternatively, the blocking buffer can be aspirated after step 3 and the plates can be dried under vacuum. When sealed with desiccant, the plates can be stored at 4° - 8° C for at least 2 months.

Assay Procedure

1. Dilutions of unknowns and standards should be carried out in polypropylene tubes. Add 100 µL of sample or standards in an appropriate diluent, per well. Mix by gently tapping the plate frame for 1 minute. Cover with an adhesive strip 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 biotinylated detection antibody, diluted in the appropriate diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 µL streptavidin HRP (R&D Systems, Catalog # DY998, dilute according to the directions on the vial label) to each well. Cover the plate and incubate for 20 minutes at room temperature.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 - 30 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 within 30 minutes, 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.

Calculation of Results

To calculate assay results, average the duplicate readings and subtract the zero standard optical density from the sample optical density. Create a standard curve using data reduction software capable of generating a four parameter (4P-L) curve fit. Alternatively, plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log-log paper and regression analysis may be applied to the log transformation. To determine the rat CINC-2α/β concentrations for each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding rat CINC-2α/β concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. A standard curve should be generated for each set of samples assayed.

Limitations

It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluent suggested in the above protocol may be suitable for most cell culture supernate samples. Validate diluents for specific sample types prior to use.

A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays. The protocol provided is for demonstration purposes only. The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range.

[1]rh6Ckine, rm6Ckine, rhBLC/BCA-1, rmBLC, rmC10, rhCCL28, rmCCL28, rrCINC-1, rmC-TACK, rvCMV UL146, rvCMV UL147, rmCRG-2, rhENA-78, rhEotaxin, rmEotaxin, rhFractalkine, rmFractalkine, rhGCP-2, rmGCP-2, rhGROα, rhGROβ, rhGROγ, rhHCC-1, rhI-309, rhI-Linck, rpIL-8, rhIP-10, rhI-TAC, rmI-TAC, rmJE, rmKC, rhLDGF, rhLeukotactin-1, rmLymphotactin, rmMARC, rhMCP-1, rhMCP-2, rhMCP-3, rhMCP-4, rmMCP-5, rvMCV type 2, rhMDC, rmMDC, rhMIG, rmMIG, rhMIP-1α, rmMIP-1α, rmMIP-1β, rhMIP-1δ, rmMIP-1γ, rmMIP-2, rhMIP-3α, rmMIP-3α, rrMIP-3α, rhMIP-3β, rmMIP-3β, rvMIP-I, rvMIP-II, rvMIP-III, rhMPIF-1, rhMPIF-2, rmMPIF-2, rhNAP-2, rhPARC, rhRANTES, rmRANTES, rhSDF-1α, rmSDF-1α, rhSDF-1β, rhTARC, rmTCA-3, rhTeck, rmTeck