

ORDERING INFORMATION

Catalog Number: MAB529

Clone: 110904

Lot Number: EEJ02

Size: 500 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: mouse CCL17

Immunogen: *E. coli*-derived rmCCL17

Ig class: rat IgG_{2A}

Recommended Applications:
ELISA capture
Neutralization of bioactivity

Other Application:
Western blot

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with the purified, *E. coli*-derived, recombinant mouse CCL17 (rmCCL17). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. CCL17 is a CC chemokine designated TARC. CCL17 binds and activates CCR-4, a chemokine receptor primarily expressed on T cells.

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 rmCCL17 bioactivity and for use in mouse CCL17 sandwich ELISAs.

Applications

Neutralization of Mouse CCL17 Bioactivity - The exact concentration of antibody required to neutralize rmCCL17 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 Dose₅₀ (ND₅₀)** 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.

Figure 1

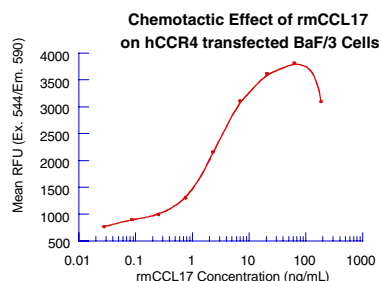
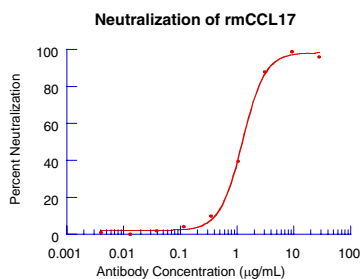


Figure 1: Recombinant mouse CCL17 chemoattracts BaF/3 cells transfected with hCCR4. The number of cells that have migrated through to the lower chamber are quantified using Resazurin (R&D Systems, Catalog # AR002) staining. The ED₅₀ for this effect is typically 2 - 8 ng/mL.

Figure 2: Approximately 0.75 - 3.0 µg/mL of the antibody will neutralize 50% of the bioactivity due to 20 ng/mL of mouse CCL17.

Figure 2



Western Blot - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect mouse CCL17. Using a colorimetric detection system, the detection limit for rmCCL17 is approximately 50 ng/lane under non-reducing conditions. Use of this antibody under reducing conditions is not recommended. Chemiluminescent detection with WesternGlo Chemiluminescent Detection Substrate (R&D Systems, Catalog # AR004) will increase sensitivity by 5 to 50 fold. In this application, the use of anti-mouse CCL17 monoclonal antibody, R&D Systems, Catalog # MAB5291, is recommended.

ELISA Capture - This antibody can be used as a capture antibody in a mouse CCL17 ELISA in combination with biotinylated, rmCCL17 affinity purified polyclonal detection antibody (Catalog # BAF529). 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. Recombinant protein standards should be titrated to establish a suitable standard curve. For this antibody pair, a two-fold dilution series starting at 2 ng/mL is suggested.

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

ELISA Protocol

Solutions Required

- **Wash Buffer** - 0.05% Tween 20 in PBS, pH 7.4
- **Diluent** - 1% BSA in PBS, pH 7.4.
- **Substrate Solution** - 1:1 mixture of Color Reagent A (H₂O₂) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY998)
- **Stop Solution** - 1 M H₂SO₄

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 and 0.05% NaN₃ 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 # DY999, 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. 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.