Certificate of Analysis for NR-58934

Monoclonal Anti-Ferret CD64 Antigen, Clone F3.4F3 (produced *in vitro*)

Catalog No. NR-58934

Product Description:

Antibody Class: IgG1κ

Mouse monoclonal antibody prepared against the ferret *(Mustela putorius furo)* CD64 (FcγRI) antigen was purified from clone F3.4F3 hybridoma supernatant using protein G affinity chromatography. The B cell hybridoma was generated by the fusion of P3X63Ag8.653 mouse myeloma cells with splenocytes from BALB/c mice immunized with recombinant ferret CD64 protein.

Lot: 70058234 **Manufacturing Date: 14OCT2021**

TEST	SPECIFICATIONS	RESULTS
Antibody Class Determination	IgG1κ	IgG1κ
SDS-PAGE Analysis	Correct molecular weight (MW) for heavy and light chains; Report purity	Correct molecular weight (MW) for heavy and light chains (Figure 1); > 95% pure
Concentration by Nanodrop	~ 1 mg/mL	1.0 mg/mL
Amount per Vial	Report results	0.025 mg
Functional Activity Western blot[1] ELISA[2] Flow cytometry[3]	 Reactive Reactive Reactive	 Reactive (Figure 2) Reactive (Figure 3) Reactive (Figure 4)
Endotoxin Content	Report results	64.72 EU/mL
Mycoplasma Contamination DNA detection by PCR	 None detected	 None detected
Sterility	0.2 µm filter-sterilized	0.2 µm filter-sterilized

[1]Recombinant CD64 antigen and BSA were used for western blot analysis. Goat anti-mouse IgG conjugated to HRP was used as the detection antibody and chemiluminescent development was applied.

[2]Recombinant CD64 antigen and BSA were used for Direct Binding ELISA. Plates were coated with 10 µg/mL of CD64 antigen or BSA and dilutions of NR-58934 were added to the wells. Goat anti-mouse IgG conjugated to HRP was used as the detection antibody. Colorimetric detection was performed using 3,3',5,5'-Tetramethyl benzidine (TMB) substrate.

[3]FiTEC.FrtCD64.E3 (ferret immortalized tracheal epithelial cells) cell clones expressing ferret CD64 were stained with dilutions of NR-58934 as primary antibody and goat anti-mouse IgG conjugated with Alexa488 as secondary antibody.

Figure 1: SDS-PAGE Analysis

Lane 1: MW Markers
Lane 2: NR-58934 (10 µg)

Figure 2: Western Blot Analysis

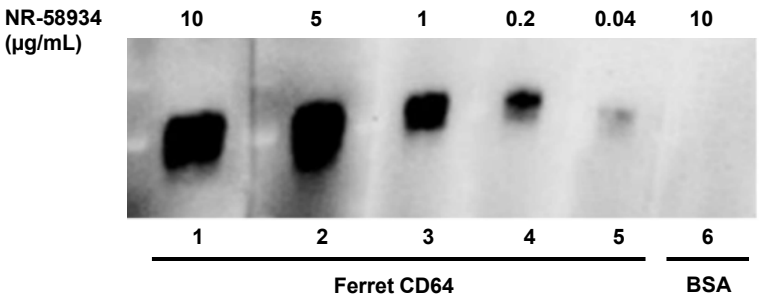

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Figure 3: ELISA

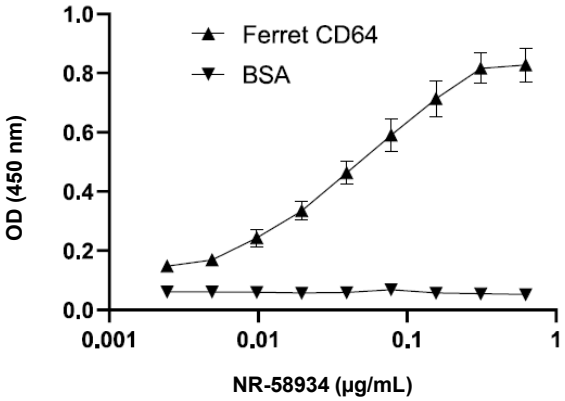
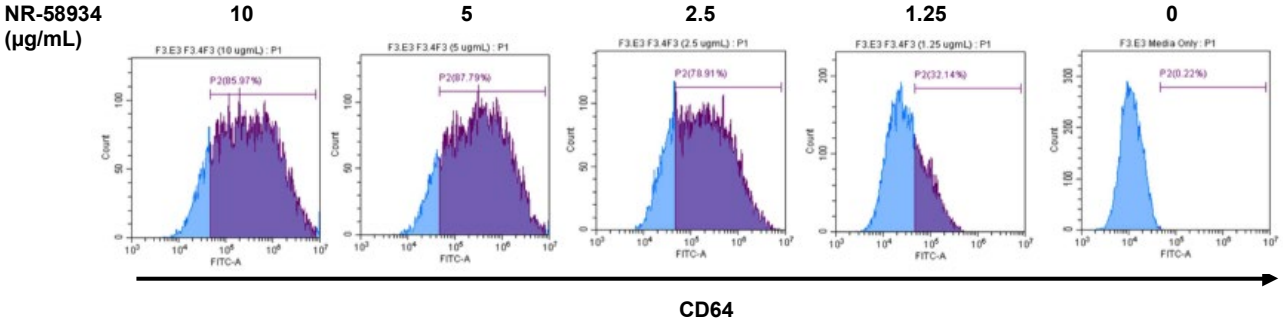


Figure 4: Flow Cytometry



/Sonia Bjorum Brower/
Sonia Bjorum Brower 09 MAR 2023
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