

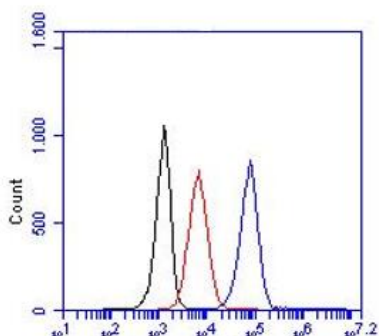
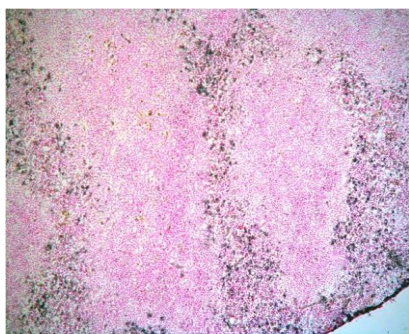
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

| | | | |
|-----------------------|---|----------------------|-----------|
| Product name | LY-6G/-6C, Mouse, clone NIMP-R14, FITC conjugated | | |
| Catalog number | HM1039F-20UG | | |
| Lot number | - | Expiry date | - |
| Volume | 200 µl | Amount | 20 µg |
| Formulation | 0.2 µm filtered in PBS+1%BSA+0.02%Na ₃ | Concentration | 100 µg/ml |
| Host Species | Rat IgG2b | Conjugate | FITC |
| Endotoxin | N.A. | Purification | Protein G |
| Storage | 4°C | | |

Application notes

| | IHC-F | IHC-P | IF | FC | FS | IA | IP | W |
|-------------|-------|-------|----|-------|-----------|----|----|---|
| Reference # | 6,10 | 4,5,9 | | 1,3,7 | 2,3,5,6,8 | 1 | | |
| Yes | • | • | | • | • | • | | |
| No | | | | | | | | |
| N.D. | | | • | | | | • | • |

N.D. = Not Determined; IHC = Immuno histochemistry; F = Frozen sections; P = Paraffin sections; IF = Immuno Fluorescence; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IP = Immuno Precipitation; W = Western blot



IHC-F: frozen sections of mouse spleen. HM1039 was used in a concentration of 5 µg/ml.

FC: detection of Ly-6G/-6C in RAW cells. Red, black and blue line represent the isotype control, cells only and HM1039PE with a concentration of 10 µg/ml, respectively.

Dilutions to be used depend on detection system applied. It is recommended that users test the reagent and determine their own optimal dilutions. The typical starting working dilution is 1:50.

- IHC-F: Tissue was fixed with acetone (Ref 6).
- IHC-P: Blocking with 20% normal rabbit serum (Ref 4). Use a mild or no antigen retrieval method.
- FC: 5 x 10⁵ cells were incubated with 10 µg/ml antibody (Ref 3).
- FS: Neutrophil depletion. Mice were treated with NIMP-R14 given intraperitoneally at a dose of 1mg, 6h before infection (Ref 5).
- Positive control: Mouse neutrophils; Negative control: Mouse Thymocytes.

General Information

Description

The monoclonal antibody NIMP-R14 is highly specific for murine Ly-6G and Ly-6C. The Ly-6G/-6C locus encodes a family of Ly-6 proteins including Ly-6G and Ly-6C. Ly-6 antigens have a molecular weight between 15,000 and 18,000. Ly6G is together with Ly6c a component of the myeloid differentiation antigen Gr-1. Ly6G a GPI-anchored protein and is a good marker of peripheral neutrophils. Although predominantly presents on neutrophils, it is also expressed on a subset of eosinophils, differentiating pre-monocytes and plasmacytoid dendritic cells. Ly6C is a monocyte/macrophage and endothelial cell differentiation antigen regulated by interferon gamma, and may play a role in the development and maturation of lymphocytes. It is expressed on bone marrow cells, monocytes/macrophages, neutrophils, endothelial cells, and T cell subsets. Expression of Gr-1 in bone marrow correlates with granulocyte differentiation and maturation. However, the physiological role of Ly6G alone remains still unclear. The monoclonal antibody NIMP-R14 has been successfully used to stain polymorphonuclear (PMN) cells and monocytes for fluorescent activated cell sorting and in frozen and paraffin sections. Treatment with antibodies in vivo leads to neutropenia and has inhibitory effect on local

immune responses. Furthermore, it has been shown to be useful for depletion of neutrophils in mice. It depletes neutrophils as soon as 6 hours after injection and up to 6 days.

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|------------------------------|---|
| Immunogen | Purified BALB/c mouse neutrophils |
| Aliases | Lymphocyte antigen 6 complex locus protein G6c, myeloid differentiation antigen Gr1 |
| Gene | Gene name: Ly6g |
| References | <ol style="list-style-type: none">1. Lopez, A et al; Differentiation antigens on mouse eosinophils and neutrophils identified by monoclonal antibodies. <i>Brit J Haematology</i> 1984, <i>57</i>: 4892. McLaren, D et al; Schistosoma mansoni: evidence that immunity in vaccinated and chronically infected CBA/Ca mice is sensitive to treatment with a monoclonal antibody that depletes cutaneous effector cells. <i>Parasite Immunol</i> 1987, <i>9</i>: 6673. Van Lent, P et al; Monocytes/macrophages rather than PMN are involved in early cartilage degradation in cationic immune complex arthritis in mice. <i>J Leukoc Biol</i> 1997, <i>61</i>: 2674. Lubberts, E et al; Adenoviral vector-mediated overexpression of IL-4 in the knee joint of mice with collagen-induced arthritis prevents cartilage destruction. <i>J Immunol</i> 1999, <i>163</i>: 45465. Tacchini-Cottier, F et al; An immunomodulatory function for neutrophils during the induction of a CD4+ Th2 response in BALB/c mice infected with Leishmania major. <i>J Immunol</i> 2000, <i>165</i>: 6286. Vries de, B et al; Complement factor C5a mediates renal ischemia-reperfusion injury independent from neutrophils. <i>J Immunol</i> 2003, <i>170</i>: 38837. Nagendra, S et al; Absence of cross-reactivity between murine Ly-6C and Ly-6G. <i>Cytometry A</i> 2004, <i>58</i>: 1958. Navarini, A et al; Innate Immune-induced depletion of bone marrow neutrophils aggravates systemic bacterial infections. <i>PNAS</i> 2009, <i>106</i>: 71079. Paust, H et al; Chemokines play a critical role in the cross-regulation of Th1 and Th17 immune responses in murine crescentic glomerulonephritis. <i>Kidney International</i>, 2012.10. Gao, N et al; Matrix Metalloproteinase-13 as a Target for Suppressing Corneal Ulceration Caused by Pseudomonas aeruginosa Infection. <i>J Infect Dis.</i> 2015 <i>212</i>:116 |
| Storage&stability | Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year. |
| Precautions | For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product. |

We hereby certify that the above-stated information is correct and that this product has been successfully tested by the Quality Control Department. This product was released for sale according to the existing specifications. This document has been produced electronically and is valid without a signature.

Approved by Manager of QC
Brenda Teunissen

Date
26/10/2020

Do you have any questions or comments regarding this product? Please contact us via support@hycultbiotech.com.