

Datasheet for 200-4199**B-Phycoerythrin Antibody****Overview**

Description:	Anti-B-PHYCOERYTHRIN (RABBIT) Antibody (BULK ORDER) - 200-4199
Item No.:	200-4199
Size:	50 mg
Applications:	ELISA, IF, IHC, Multiplex, Other
Reactivity:	Phycoerythrin
Host Species:	Rabbit

Product Details

Background:	Anti-B-Phycoerythrin Antibody detects the B-PE phycoerythrin protein. Phycoerythrin is a red protein from the light-harvesting phycobiliprotein family, present in cyanobacteria, and red algae. Phycoerythrin is organized in a hexameric structure of alpha and beta chains, covalently binding chromophores called phycobilins. R-Phycoerythrin and B-Phycoerythrin are among the brightest biological fluorescent dyes and are commonly used as a fluorescent dye in many assays and is frequently conjugated to antibodies as a reporter molecule. The spectral properties of B-Phycoerythrin are absorption at 545 nm and emission at 572 nm.
Synonyms:	rabbit anti-B-Phycoerythrin Antibody, rabbit anti B-Phycoerythrin, rabbit anti Phycoerythrin
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	cpeA, cpeB
Reactivity:	Phycoerythrin
Immunogen Type:	Native Protein
Immunogen:	Phycoerythrin [Porphyridium creuentum]

Purity/Specificity:	Anti-B-Phycoerythrin Antibody is an IgG fraction antibody purified from monospecific antiserum by a multi-step process which includes delipidation, salt fractionation and ion exchange chromatography followed by extensive dialysis against the buffer stated above. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum as well as purified and partially purified Phycoerythrin [Porphyridium creurentum].
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Application Details

Tested Applications:	ELISA
Suggested Applications:	IF, IHC, Multiplex, Other (Based on references)
Application Note:	Anti-B-Phycoerythrin antibody has been tested by ELISA and is suitable for western blot, immunohistochemistry, immunomicroscopy and flow cytometry or FACS analysis as well as other antibody based fluorescent assays requiring lot-to-lot consistency.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000 - 1:100,000
IHC:	1:1,000 - 1:5,000
WB:	1:2,000 - 1:10,000

Formulation

Physical State:	Lyophilized
Concentration:	10.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None
Reconstitution Volume:	5.0 mL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

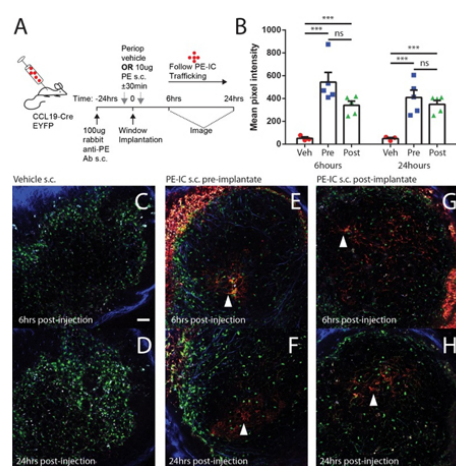
Shipping & Handling

Shipping Condition:	Ambient
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Storage Condition: Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

Expiration: Expiration date is one (1) year from date of receipt.

Images

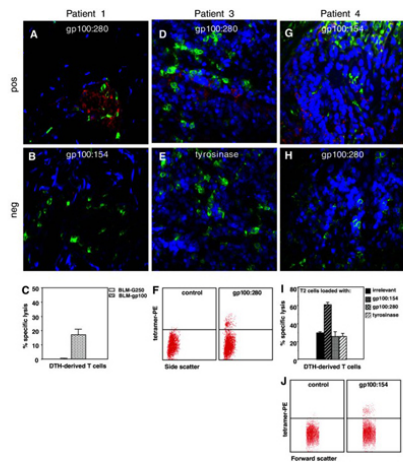


Immunofluorescence Microscopy

Perioperative PE-IC immunization reveals intact lymphatics in CLNW operated mice.

(A) Schematic overview of experimental approach for CCL19-Cre EYFP mice, preoperative s.c. injection with rabbit anti-PE Ab, followed by either immediately (30 min) pre- or post-CLNW operation injections of either 10 ug PE or vehicle. iLN were then imaged for PE signal at 6 and 24 hr post-implantation. (B) Quantification of veh and PE injected LN at 6 and 24 hr reveals rapid uptake of PE onto the FDC network within each follicle in PE injected animals (***) represents $p < 0.0001$). There was no significant difference (ns) between PE uptake in pre vs post-operation PE injection confirming the integrity of the lymphatics during surgical implantation of CLNW. (C) Representative follicles following veh at 6 hr and (D) 24 hr post-implantation time. Scale bar = 100 um. (E, F) As in (C, D) but for pre-implantate injection of PE. Arrowheads note the reticular pattern of PE-IC labeling of FDC. (G, H) As in (C, D) but for post-implantate injection of PE.

Fig2-SFig1. PMID: 30066671

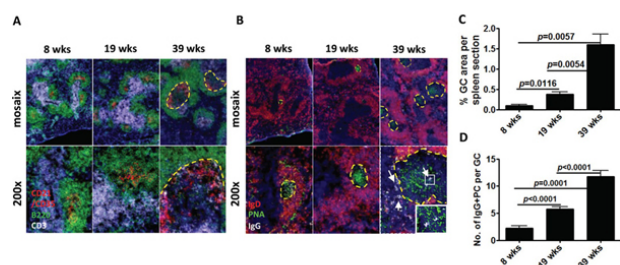


Immunofluorescence Microscopy

Comparison of MHC class I tetramer staining in situ and MHC class I tetramer staining of cell suspensions or cytotoxic activity of cultured T cells of same DTH biopsies from melanoma patients vaccinated with peptide-loaded DC. Cryosections stained with MHC class I tetramers (red), anti-CD8 (green) and DAPI (blue). DTH biopsy (DC loaded with gp100:280) from patient 1. a Positive HLA-A2.1-gp100280 tetramer staining (red). b HLA-A2.1-gp100154 tetramer staining. c Chromium release assay showing the lysis of HLA-A2.1-positive BLM cells, transfected with gp100 and not the control transfectant G250 by T cells cultured from the DTH biopsy. DTH biopsy (DC loaded with 3 peptides and KLH) from patient 3. d Positive HLA-A2.1-gp100280 tetramer staining. e Cryosection from the same area as shown in d stained with HLA-A2.1-tyrosinase tetramer. f Tetramer analysis by flow cytometry of DTH-derived T cells. Depicted is the side scatter on the x-axis (double staining with CD8 FITC might interfere with the tetramer staining) and on the y-axis MHC class I tetramer PE staining. DTH biopsy (DC loaded with 3 peptides and KLH) from patient 4. g Positive HLA-A2.1-gp100154 tetramer staining. h Cryosection from the same area as shown in g stained with HLA-A2.1-gp100280 tetramer. i Chromium release assay showing the lysis of HLA-A2.1-positive T2 cells, loaded with gp100:154 and not T2 cells loaded with irrelevant, gp100:280 or tyrosinase peptides by T cells cultured from the DTH biopsy. j Tetramer analysis by flow cytometry of DTH-derived T cells. Depicted is the forward scatter on the x-axis (double staining with CD8 FITC might interfere with the tetramer staining) and on the y-axis MHC class I tetramer PE staining.

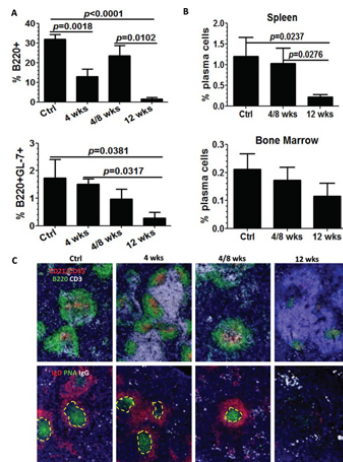
Fig 3.

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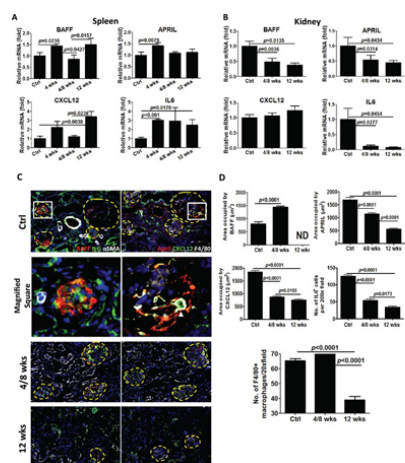
Immunofluorescence Microscopy

A. Representative pictures from spleens (n=5) of each group are shown. 4 μ m spleen frozen sections were probed with antibodies against CD21-CD35 (red), B220 (green) and CD3 (white) to reveal T cell spatial distribution and FDC networks. B. Antibodies against IgD (red), peanut agglutinin (green) and IgG (white) were used to detect bona fide GC and isotype-switched PC, arrow indicates isotype-switched PC. The mosaic figures in the top rows are a compilation of 16 200X images. GC is outlined with yellow dashed line. White arrows point to isotype-switched IgG+ cells. Square area is enlarged and put on left bottom. Representative data from two experiments with similar results are shown. C. GC area was measured in blinded fashion by IPLab 4.0 software. Bars show mean $\pm$ SEM. p value is calculated with unpaired t-test. Average GC size and number were also significantly higher as mice aged (size: $p=0.005$ 39 wk vs. 8 wk and $p=0.0001$ 39 wk vs. 19 wk; number: $p=0.006$ 39 wk vs. 8 wk and $p=0.03$ 19 wk vs. 8 wk). n=3 8 wk, n=4 19 wk, n=5 39 wk mice. D. IgG+ plasma cells in GCs were measured in blinded fashion. Bars show mean $\pm$ SEM. p value is calculated with unpaired t-test. Fig 2. PMID: 24574498



Immunofluorescence Microscopy

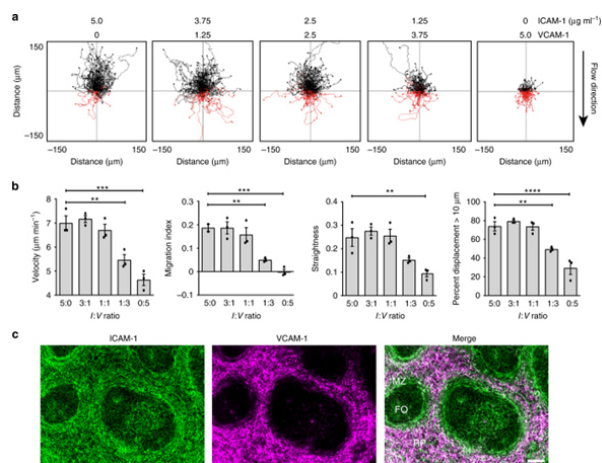
As described above, four groups including control mice treated with control IgG (Ctrl) (n=6), mice treated with anti-mCD20 antibody for 4 weeks (4 wks) (n=8), mice with 8 weeks reconstitution after 4 weeks treatment with anti-mCD20 antibody (4/8 wks) (n=6), and mice treated with anti-mCD20 antibody for 12 weeks (n=4) were sacrificed. Tissue and lymphocytes were collected and analyzed by flow cytometry and immunohistochemistry. A. Percentage of residual B cells after BCD via flow cytometry expression of B220 (upper) and GL-7 (lower) in lymphocytes in spleen. B. Percentage of residual plasma cells in total cells after treatment with anti-mCD20 antibody. Plasma cells were enumerated by flow cytometry as CD138+k light chain+ cells. Bars show mean±SEM. C. Changes in the splenic architecture associated to long-term CD20 depletion. 4 µm spleen frozen sections were stained with antibodies to detect follicular dendritic cells (CD21-CD35, red), B cells (B220, green) and T cells (CD3, white). Germinal centers and plasma cells were visualized with antibodies against IgD (red), peanut agglutinin (green) and IgG (white). GCs are outlined with dashed yellow lines. Representative of n=5 mice per group. The average number of IgG+ cells counted in 5–10 randomly selected 200x fields significantly decreased after 12 wks BCD therapy (p=0.026). Fig 5. PMID: 24574498



Immunofluorescence Microscopy

Total RNA was isolated from spleens (A) and kidney (B) from control group (n=8 spleen, n=12 kidney), mice treated for 4 weeks (n=5 only spleen), 4/8 week mice (n=5 spleen and n=6 kidney), and mice treated for 12 weeks (n=4 spleen and kidney). qPCR was performed as described in Figure 3 and the Methods. Levels of expression for each gene were first normalized to GAPDH and then compared to the levels of expression in the control group, according to the $\Delta\Delta CT$ method. Representative data from two experiments with similar results is shown. C. Decrease in PC survival factor expression in the kidney after long-term CD20 depletion. 4 μ m kidney frozen sections were stained with antibodies to detect BAFF (red), APRIL (red), IgG (green), CXCL12 (green) and IL6 (data not shown). Macrophages were visualized with antibodies against F4/80 (white). α -SMA (white) was used for detecting myoepithelial cells. Glomeruli are outlined with dashed yellow lines. The square area stained with BAFF/IgG/ α -SMA is magnified and shown on left middle. The square area stained with APRIL/CXCR12/F4/80 is magnified and shown on right middle. The arrow highlights a CXCL12 expressing macrophage. The asterisk highlights a stromal cell co-expressing APRIL and CXCL12. Representative of n=5 mice per group, quantitated in D. D. Quantitation of F4/80+ macrophages, BAFF, APRIL, CXCR12 and IL6 expression was performed by morphometric analysis and reveals a significant decrease after BCD (ND=non detected). Bars show mean $\pm$ SEM. p values are calculated by unpaired t- test. Fig 6.

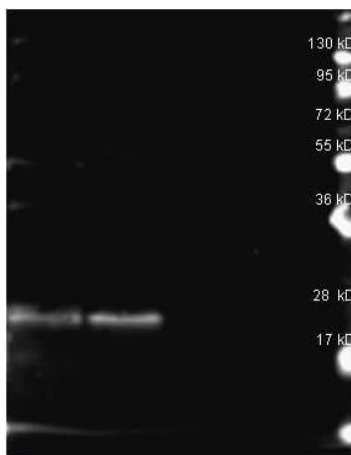
PMID: 24574498



Immunofluorescence Microscopy

MZBs migrate up the flow on ICAM-1 but not on VCAM-1. **a** Representative track plots of MZB migration up the flow (8 dyn cm⁻²; direction indicated by arrow) with coating (ICAM-1 or VCAM-1) amounts shown above. **b** Quantification of velocity, straightness, migration index, and % of cells with displacement >10 μm for the ICAM-1 to VCAM-1 (I/V) ratios shown above in **a**. All four graphs show different parameters from the same set of experiments; bars show mean ± SEM. Data are from three experiments with one mouse each. Symbols in one condition group denote the three replicates and represent the average of 100–200 cells from each individual mouse. **c** Microscopy of ICAM-1 (green), VCAM-1 (magenta), and the merge (white) in the spleen, showing follicles (FO), marginal zone (MZ), and red pulp (RP). Data represent two experiments with one spleen each. Scale bar; 100 μm. **P < 0.01, ***P < 0.001, ****P < 0.0001, one-way ANOVA with Dunnett post hoc tests comparing all ICAM-1/VCAM-1 mixtures to ICAM-1 alone (5:0).

Fig 2. PMID: 29273735



Western Blot

Rockland Rabbit anti B-Phycoerythrin antibody (200-4199 lot 25411) was used to detect B-Phycoerythrin under reducing (R) conditions. Reduced samples of purified Phycoerythrin contained 4% BME and were boiled for 5 minutes. Samples of ~1μg of protein per lane were run by SDS-PAGE. Protein was transferred to nitrocellulose and probed with 1:1000 dilution of anti B-Phycoerythrin antibody (200-4199 lot 25411 ON 4 C) and detection was using Dylight 649 conjugated Donkey anti rabbit (611-743-127 lot 20831 1:10K 1.5 hr RT in MB-070) and imaged on the BioRad VersaDoc System.

References

- Pirgova G et al. Marginal zone SIGN-R1+ macrophages are essential for the maturation of germinal center B cells in the spleen. *Proc Natl Acad Sci USA*. (2020)
- Kerry Tedford et al. The opposing forces of shear flow and sphingosine-1-phosphate control marginal zone B cell shuttling. *Nat Commun*. (2017)
- Rodda LB et al. Phenotypic and Morphological Properties of Germinal Center Dark Zone Cxcl12-Expressing Reticular Cells. *J Immunol*. (2015)
- Wang W et al. Long-term B cell depletion in murine lupus eliminates autoantibody-secreting cells and is associated with alterations in the kidney plasma cell niche. *J Immunol*. (2014)
- Green JA et al. The sphingosine 1-phosphate receptor S1P₂ maintains the homeostasis of germinal center B cells and promotes niche confinement. *Nat Immunol*. (2011)
- de Vries IJM et al. In situ detection of antigen-specific T cells in cryo-sections using MHC class I tetramers after dendritic cell vaccination of melanoma patients. *Cancer Immunol Immunother*. (2007)

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.