OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM39032PU-N

HLA Class II DR Mouse Monoclonal Antibody [Clone ID: BRA30]

Product data:

Product Type:	Primary Antibodies
Clone Name:	BRA30
Applications:	FC, IF, IHC
Recommended Dilution:	Clone BRA30 can be applied in **Flow Cytometry** for the enumeration of B cells and monocytes in peripheral blood, the study of activated T cells and the characterization of leukemia's and lymphomas. Suitable also for I**mmunohistochemistry using cytospots or frozen tissue sections**. Clone BRA30 can also be used for analysis of bone marrow samples and may be used for immunoprecipitation of HLA-DR antigens. Flow cytometry: please see "***Protocols***" below. Labelled reagent is effectively formulated for direct immunofluorescent staining. ***Reommended Use:*** 10 µl of antibody solution for 10^6 leukocytes.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal

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Specificity: The antibody clone BRA30 is specific to a non-polymorphic determinant of the HLA-DR antigen (36 kD).
BRA30 does not cross react with HLA-DQ or HLA-DP antigens.

Testing by flow cytometry using a 'lyse-wash' method on whole blood from healthy donors showed the following values expressed in terms of % of the total lymphocyte count:

Product code: AM39032FC-N (anti-HLA-DR FITC)
n: 10
Mean % positive: 15,38
S.D.: 2,76
% CV: 17,94

Product code: AM39032RP-N (anti-HLA-DR PE)
n: 10
Mean % positive: 17,89
S.D.: 2,73
% CV: 15,25

Formulation: 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.3
State: Aff - Purified
State: Liquid purified Ig fraction
Stabilizer: 0.2% BSA
Preservative: 0.09% Sodium Azide
Label: **Cat. No. Label EX-max (nm) / EM-max (nm):**
AM39032FC-N FITC 488 / 519
AM39032RP-N 488, 532 / 578
AM39032PU-N Pure . /

Concentration: lot specific

Purification: Affinity Chromatography

Conjugation: Unconjugated

Storage: Store the antibody undiluted at 2-8°C.
Fluorochrome labelled product is photosensitive and should be protected from light.

Stability: Shelf life: one year from despatch.

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Background: HLA-DR is a MHC Class II antigen expressed on dendritic cells, B cells, monocytes, macrophages, myeloid and erythroid precursors and some epithelial cells. MHC class II antigens are also expressed on activated T cells.
Expression of MHC class II antigens is regulated by cytokines such as IFN-gamma, which also induces expression on fibroblasts, epithelial and endothelial cells. Certain HLA Class II molecules are associated with autoimmune diseases such as coeliac disease, insulin-dependent diabetes mellitus, rheumatoid arthritis and multiple sclerosis. MHC class II molecules are heterodimers of non-covalently associated a and b chains. MHC II on antigen presenting cells bind and present processed peptide antigens, which are then recognized by the T cell receptor on CD4+ cells.

Synonyms: HLA-DR, HLA class II histocompatibility antigen DR, MHC class II antigen DR

Note: 1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC. When populations overlap, the percentage of positive cells using a selected marker can be affected by the choice of fluorescent label.
2. Use of monoclonal antibodies in patient treatment can interfere with antigen target recognition by this reagent. This should be taken into account when samples are analyzed from patients treated in this fashion.
3. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

Protocol: Flow cytometry method for use with labelled (FITC, R-PE, APC, PerCP or PerCP-Cy5.5) monoclonal antibodies
1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
2. Add to each tube 10 µl of labelled monoclonal antibody. (Appropriate mouse Ig isotype control samples should always be included in any labelling study).
Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for 15 minutes at room temperature in the dark.
4. Add 100 µl of a lyse reagent.
5. Incubate for 10 minutes at room temperature in the dark.
6. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
7. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
8. Remove the supernatant and resuspend the cells in 200 µl of PBS.
9. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

Flow cytometry method for use with dual and triple combinations
1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
For combinations with anti-kappa and/or anti-lambda Ig see **Application note** below.
2. Add to each tube 20 µl of labelled monoclonal antibody combination.
(Appropriate mouse Ig isotype control samples should always be included in any labelling

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study).
3. Vortex the tube to ensure thorough mixing of antibody and cells.
4. Incubate the tube for 15 minutes at room temperature in the dark.
5. Add 100 µl of a lyse reagent and mix immediately.
6. Incubate for 10 minutes at room temperature in the dark.
7. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
8. Centrifuge the labelled cell suspension for 2 minutes at 1000 x g.
9. Remove the supernatant and resuspend the cells in 200 µl of PBS.
10. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

Application note for anti-kappa and/or anti-lambda Ig combinations

Add 2 ml of PBS containing 0.001% (v/v) Heparin (prewarmed to 37°C) to the cell suspension Vortex, centrifuge (2 min at 300x g) and discard the supernatant.
Repeat this step twice.
Resuspend the pelleted blood cells in 100 µl PBS, pH 7.2, containing 0.001% (v/v) Heparin.

Flow cytometry method for use with purified monoclonal antibodies

1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
2. Add to each tube 10 µl of purified monoclonal antibody. (Appropriate mouse Ig isotype control samples should always be included in any labelling study).
Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for 15 minutes at room temperature in the dark.
4. Wash the labelled cells by adding 2 ml of PBS containing 0.001% (v/v) Heparin, vortexing and centrifuging (2 min 1000 x g) and discard the supernatant.
5. Add 50 µl of appropriate dilution of F(ab)2 Rabbit Anti Mouse IgG fluorescent conjugate (e.g.FITC or R-PE) in PBS containing 0.001% (v/v) Heparin to the tube. It is recommended that the tube is protected from light.
6. Mix by vortexing and incubate for 15 minutes at room temperature in the dark.
7. Add 100 µl of a lyse reagent and mix immediately.
8. Incubate for 10 minutes at room temperature in the dark.
9. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
10. Centrifuge the labelled cell suspension for 2 minutes at 1000 x g.
11. Remove the supernatant and resuspend the cells in 200 µl of PBS.
12. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

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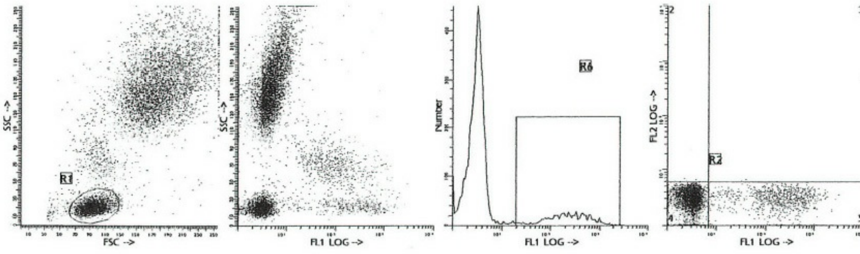
Product images:



Representative Data Staining with clone BRA30 (anti-HLA-DR) monoclonal antibody is illustrated by flow cytometry of normal blood cells. Direct staining was performed using 10 l FITC-conjugated antibody with 100 l blood sample.

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