

Technical Data Sheet

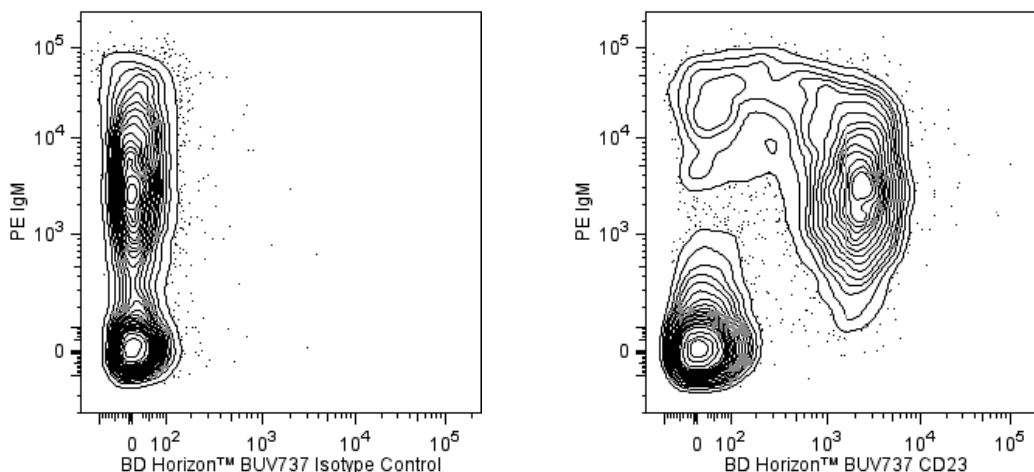
BUV737 Rat Anti-Mouse CD23**Product Information**

| | |
|-------------------------|---|
| Material Number: | 564436 |
| Alternate Name: | FcεRII; Fc-epsilon-RII; Fcεr2a; Ly-42; Low-affinity IgE receptor; Fcεr2 |
| Size: | 50 µg |
| Concentration: | 0.2 mg/ml |
| Clone: | B3B4 |
| Immunogen: | FcεR isolated from the mouse B hybridoma line O1.2B2 |
| Isotype: | Rat (LOU) IgG2a, κ |
| Reactivity: | QC Testing: Mouse |
| Storage Buffer: | Aqueous buffered solution containing ≤0.09% sodium azide. |

Description

The B3B4 monoclonal antibody specifically binds to CD23, the low affinity IgE Fc receptor (FcεRII) expressed on mature resting conventional B lymphocytes, but not on B-1 cells (CD5+ B cells) or T lymphocytes. It does not react with high-affinity IgE receptors, as demonstrated on mouse mast cell lines. The regulation of CD23 surface expression on activated B cells appears to be complex, depending upon the mode of activation and the presence of cytokines. IgE synthesis is negatively regulated by CD23, and CD23 expression is upregulated on splenocytes in the presence of IgE. CD23 is also upregulated on follicular dendritic cells in the lymph nodes of immunized mice, and a subset of splenic dendritic cells expresses CD23. The B3B4 antibody abrogates antigen-specific IgE-dependent modulation of immune responses in normal mice. This monoclonal antibody also blocks IgE binding and eosinophil infiltration in the lung of immunized mice. Different *in vivo* results have been obtained when using the intact B3B4 antibody or the F(ab')₂ fragments. B3B4 mAb does not cross-react with rat or human IgE Fc Receptor.

The antibody was conjugated to BD Horizon BUV737 which is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This dye is a tandem fluorochrome with an Ex Max near 350 nm and an Em Max near 737 nm. BD Horizon Brilliant BUV737 can be excited by the ultraviolet laser (355 nm) and detected with a 740/35 nm filter. Due to the excitation of the acceptor dye by the red laser line, there may be significant spillover into red laser detectors with filters in the 700-720 nm range.



Two-color flow cytometric analysis of CD23 expression on mouse splenocytes. BALB/c mouse splenic leucocytes were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with PE Anti-Mouse IgM antibody (Cat. No. 553517) and either BD Horizon™ BUV737 Rat IgG2a, κ Isotype Control (Cat. No. 564294; Left Panel) or BD Horizon BUV737 Rat Anti-Mouse CD23 antibody (Cat. No. 564436; Right Panel). Two-color flow cytometric contour plots showing the correlated expression of CD23 (or Ig Isotype control staining) versus IgM were derived from gated events with the forward and side light-scatter characteristics of viable splenic leucocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.

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564436 Rev. 2



Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon BUV737 under optimum conditions, and unconjugated antibody and free BD Horizon BUV737 were removed.

Application Notes

Application

Flow cytometry

Routinely Tested

Recommended Assay Procedure:

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to BD CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and BD CompBead to ensure that BD CompBeads are appropriate for your specific cellular application.

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Note: When using high concentrations of antibody, background binding of this dye to erythroid cell subsets (mature erythrocytes and precursors) has been observed. For researchers studying these cell populations, or in cases where light scatter gating does not adequately exclude these cells from the analysis, this background may be an important factor to consider when selecting reagents for panel(s).

Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|----------------|--|------------|--------|
| 554657 | Stain Buffer (BSA) | 500 mL | (none) |
| 563794 | Brilliant Stain Buffer | 100 Tests | (none) |
| 554656 | Stain Buffer (FBS) | 500 mL | (none) |
| 564294 | BUV737 Rat IgG2a, κ Isotype Control (to be replaced with 612760) | 50 µg | R35-95 |
| 555899 | Lysing Buffer | 100 mL | (none) |
| 553517 | PE Anti-Mouse IgM[a] | 0.2 mg | DS-1 |
| 553141 | Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) | 0.1 mg | 2.4G2 |
| 553142 | Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) | 0.5 mg | 2.4G2 |
| 566349 | Brilliant Stain Buffer | 1000 Tests | (none) |
| 566385 | Brilliant Stain Buffer Plus | 1000 Tests | (none) |

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. BD Horizon Brilliant Ultraviolet 737 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.
6. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
7. Please refer to <http://regdocs.bd.com> to access safety data sheets (SDS).
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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