Technical Data Sheet

# BUV737 Rat Anti-Human IL-2

## Product Information

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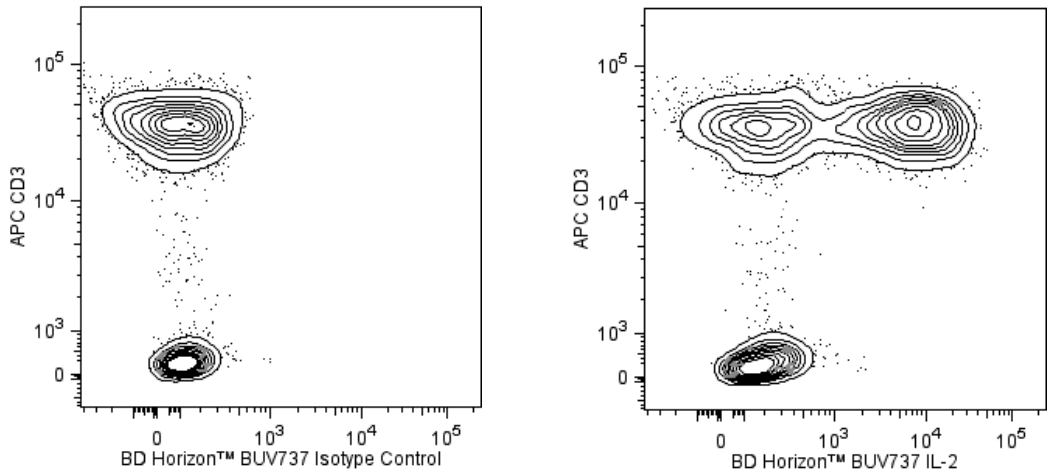
| **Material Number:** | **564446** |
| **Alternate Name:** | IL2; Interleukin-2; T-cell growth factor; TCGF |
| **Size:** | 50 Tests |
| **Vol. per Test:** | 5 µl |
| **Clone:** | MQ1-17H12 |
| **Immunogen:** | Human IL-2 Recombinant Protein |
| **Isotype:** | Rat IgG2a, κ |
| **Reactivity:** | QC Testing: Human<br>Tested in Development: Rhesus, Cynomolgus, Baboon |
| **Storage Buffer:** | Aqueous buffered solution containing ≤0.09% sodium azide. |

## Description

The MQ1-17H12 monoclonal antibody specifically binds to the multifunctional cytokine, human Interleukin-2 (IL-2). IL-2 is produced by activated T cells and has multiple functions that can affect the growth, proliferation, differentiation and survival of many different target cell types including T cells, B cells, NK cells, monocytes and macrophages. The immunogen used to generate the MQ1-17H12 hybridoma was purified recombinant human IL-2 protein. The MQ1-17H12 antibody reportedly neutralizes the biological activity of human IL-2.

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 of BD Horizon BUV395 with an Ex Max of 348-nm and an acceptor dye with an Em Max at 737-nm. BD Horizon Brilliant BUV737 can be excited by the ultraviolet laser (355 nm) and detected with a 740/35 filter. Due to the excitation of the acceptor dye by other laser lines, there may be significant spillover into channels detecting Alexa Fluor® 700-like dyes (e.g., 712/20-nm filter).

Due to spectral differences between labeled cells and beads, using BD™ CompBeads can result in incorrect spillover values when used with BD Horizon BUV737 reagents. Therefore, the use of BD CompBeads or BD CompBeads Plus to determine spillover values for these reagents is not recommended. Different BUV737 reagents (e.g., CD4 vs. CD45) can have slightly different fluorescence spillover therefore, it may also be necessary to use clone specific compensation controls when using these reagents.




***Two color flow cytometric analysis of IL-2 expression in activated human peripheral blood lymphocytes.*** *Human peripheral blood mononuclear cells were stimulated for 5 hours with Phorbol 12-Myristate 13-Acetate (PMA, Sigma P-8139; 50 ng/ml) and Calcium Ionophore A23187 (Sigma C-9275; 1 µg/ml), in the presence of BD GolgiStop™ Protein Transport Inhibitor (containing Monensin) (Cat. No. 554724). The cells were harvested, washed with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656), and fixed and permeabilized with BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution (554722).*

*The cells were then washed and stained in BD Perm/Wash™ Buffer (Cat. No. 554723) with APC Mouse Anti-Human CD3 antibody (Cat No. 555335/561810/561811) and either BD Horizon™ BUV737 Rat IgG2a, κ Isotype Control (Cat No. 564294; Left Panel) or BD Horizon BUV737 Rat Anti-Human IL-2 antibody (Cat No. 564446; Right Panel) using BD Biosciences Intracellular Cytokine Staining Protocol. The two-color flow cytometric contour plots showing correlated expression of IL-2 (or Ig Isotype control staining) versus CD3 were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.*

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## 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**

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| Intracellular staining (flow cytometry) | Routinely Tested |

**Recommended Assay Procedure:**

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).

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 563794 | Brilliant Stain Buffer | 100 Tests | (none) |
| 564294 | BUV737 Rat IgG2a, κ Isotype Control | 50 µg | R35-95 |
| 554656 | Stain Buffer (FBS) | 500 mL | (none) |
| 554724 | Protein Transport Inhibitor (Containing Monensin) | 0.7 mL | (none) |
| 554722 | Fixation and Permeabilization Solution | 125 mL | (none) |
| 554723 | Perm/Wash Buffer | 100 mL | (none) |
| 555335 | APC Mouse Anti-Human CD3 | 100 Tests | UCHT1 |
| 561810 | APC Mouse Anti-Human CD3 | 25 Tests | UCHT1 |
| 561811 | APC Mouse Anti-Human CD3 | 500 Tests | UCHT1 |
| 566349 | Brilliant Stain Buffer | 1000 Tests | (none) |
| 554657 | Stain Buffer (BSA) | 500 mL | (none) |

## Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. 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.
7. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
8. 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.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

## References


Abrams J. Immunoenzymetric assay of mouse and human cytokines using NIP-labeled anti-cytokine antibodies. *Curr Protoc Immunol.* 2001; 1:6.20-6.21. (Clone-specific: ELISA)

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24. (Clone-specific: Blocking, ELISA, Immunoprecipitation)

Mascher B, Schlenke P, Seyfarth M. Expression and kinetics of cytokines determined by intracellular staining using flow cytometry. *J Immunol Methods.* 1999; 223(1):115-121. (Clone-specific: Flow cytometry)

Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128. (Methodology: Flow cytometry)