Technical Data Sheet

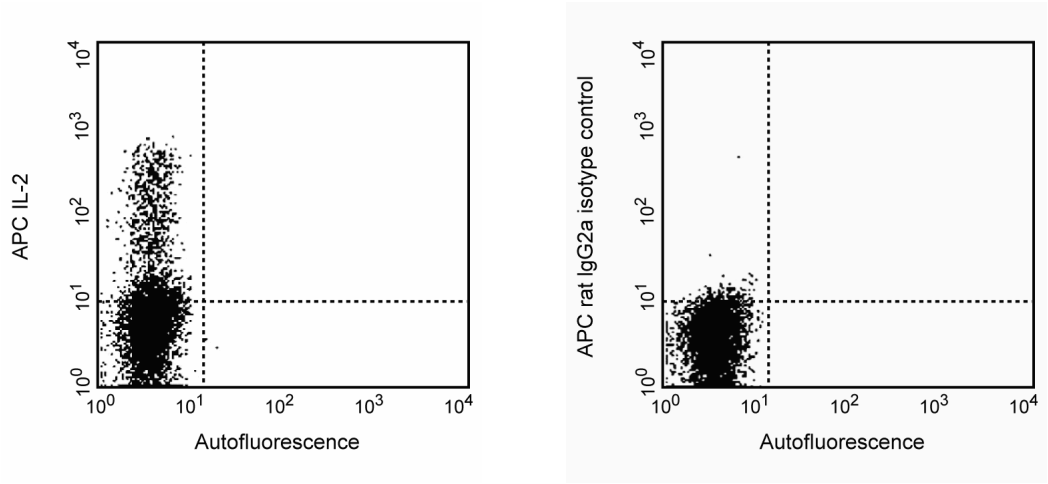
APC Rat IgG2a κ Isotype Control

Product Information

Material Number:	**554690**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	R35-95
Immunogen:	Mouse Pooled Immunoglobulin
Isotype:	Rat (LOU) IgG2a, κ
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The R35-95 hybridoma was generated by hybridization of Y3 myeloma cells with spleen cells from LOU rats immunized with mouse immunoglobulins. The R35-95 hybridoma produces rat IgG2a, κ immunoglobulin that has no measurable reactivity with mouse immunoglobulins. The R35-95 immunoglobulin was selected as an isotype control following screening for low background binding on a variety of mouse and human tissues.



Expression of IL-2 by stimulated MiCK-1 Cells. *MiCK-1 cells were stimulated for 6 hours with 5 ng PMA (Sigma, Cat. #P-8139) and 500 ng ionomycin (Sigma, Cat. #0634) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The MiCK-1 cells were fixed, permeabilized, and subsequently stained with 0.25 µg APC-rat anti-mouse IL-2 antibody (APC-JES6-5H4, Cat. No. 554429; left panel) or 0.25 µg APC-rat IgG2a isotype control (APC-R35-95; Cat. No. 554690; right panel) using the BD Pharmingen staining protocol. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking and unlabeled antibody blocking specificity controls.*

Preparation and Storage

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

The antibody was conjugated to APC under optimum conditions, and unconjugated antibody and free APC were removed.

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

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Isotype control	Routinely Tested

Neutralization Activity:

The NA/LE™ R35-95 (Cat. No. 553926) is suitable as an isotype control for rat IgG2a neutralizing antibodies.

Recommended Assay Procedure:

Immunofluroescent Staining and Flow Cytometric Analysis: The F APC-conjugated R35-95 immunoglobulin (Cat. No. 554690) are suitable rat IgG2a isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse or human cells

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for flow cytometric analysis. Use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/1 million cells). See image, right panel. The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

Product Notices

1. 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.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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. (Biology)