

## Technical Data Sheet

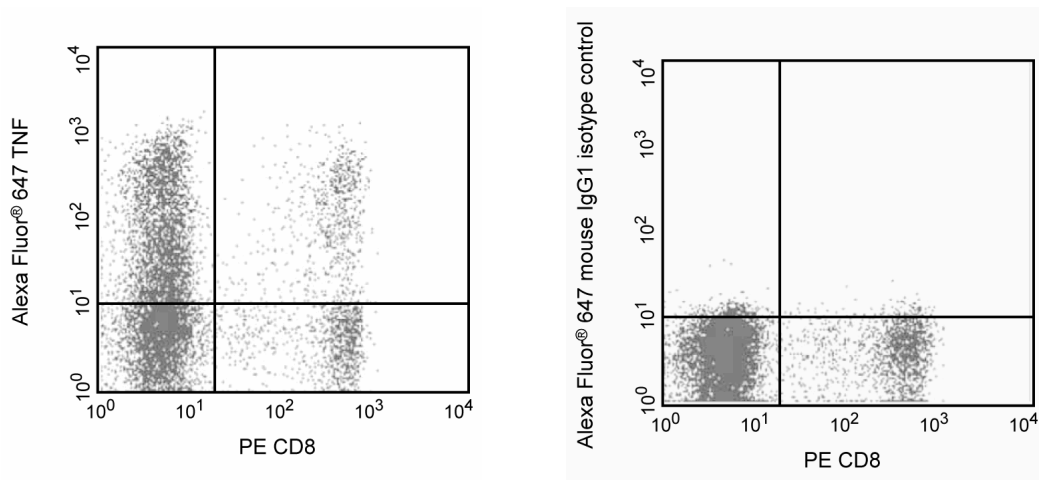
## Alexa Fluor® 647 Mouse IgG1 κ Isotype Control

## Product Information

Material Number:	557732
Size:	100 tests
Vol. per Test:	5 µl
Clone:	MOPC-21
Isotype:	Mouse IgG1, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

## Description

The MOPC-21 immunoglobulin is a mouse myeloma protein. The MOPC-21 immunoglobulin was selected as an isotype control following screening for low background on a variety of mouse and human tissues.



**Expression of TNF by stimulated human peripheral blood lymphocytes.** Human peripheral blood mononuclear cells were stimulated for 4 hours with PMA (5 ng/ml, Sigma, Cat. No. P-8139) in the presence of Brefeldin A (BD GolgiPlug™ Protein Transport Inhibitor, Cat. No. 555029). Cells were harvested, fixed, permeabilized and stained with PE-conjugated mouse anti-human CD8 (PE-RPA-T8, Cat. No. 555367) and either mouse anti-human TNF antibody (Alexa Fluor® 647-MAb11, Cat. No. 557733), (left panel) or Alexa Fluor® 647 MOPC-21 immunoglobulin (Cat. No. 557732, right panel) by using the BD Pharmingen staining protocol. To demonstrate specificity of staining the binding of Alexa Fluor® 647-MAb11 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant human TNF (Cat. No. 554618) and by preincubation of the fixed/permeabilized cells with an excess of unlabelled MAb11 antibody (Cat. No. 554510) prior to staining. Dot plots were derived from gated events with the forward and side light scatter characteristics of lymphocytes. The quadrant markers for the bivariate dot plots were set based on the autofluorescence and isotype controls.

## 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 to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

## Application Notes

## Application

Intracellular staining (flow cytometry)	Routinely Tested
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## Recommended Assay Procedure:

**Intracellular Immunofluorescent Staining Analysis:** The FITC-, PE-, APC-, PE-Cy™7-, Alexa Fluor® 488-, and Alexa Fluor® 647-MOPC-21 immunoglobulins (Cat. No. 554679, 559320, 554681, 557646, 557721, and 557732) are suitable mouse IgG1, κ isotype controls for assessing the level of background staining on paraformaldehyde fixed/saponin-permeabilized mouse or human cells for flow cytometric analysis. Use the MOPC-21 antibody at 5 µl test as recommended to serve as an isotype control.

BD Pharmingen™ Alexa Fluor® 647 Mouse IgG1 κ Isotype control, Cat. No. 557714 is recommended for non-intracellular staining.

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## Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
555367	PE Mouse Anti-Human CD8	100 tests	RPA-T8
557733	Alexa Fluor®647 Mouse Anti Human TNF	100 tests	MAb11
554618	Recombinant Human TNF	10 µg	(none)
554510	Purified Mouse Anti-Human TNF	0.1 mg	MAb11
554722	Fixation and Permeabilization Solution	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)

## Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100-µl experimental sample (a test).
2. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
5. 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.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. Cy is a trademark of Amersham Biosciences Limited.
9. An isotype control should be used at the same concentration as the antibody of interest.
10. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/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. (Clone-specific: Blocking, Flow cytometry)

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