

Technical Data Sheet

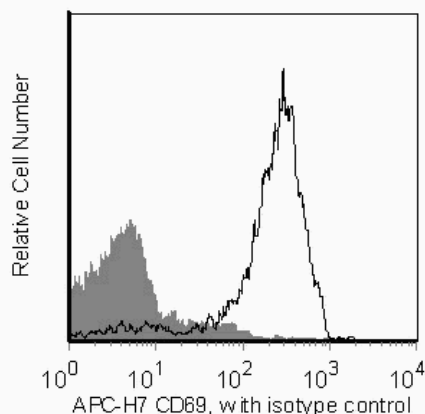
APC-H7 Mouse Anti-Human CD69

Product Information

Material Number:	560737
Alternate Name:	Very Early Activation Antigen
Size:	50 tests
Vol. per Test:	5 µl
Clone:	FN50
Isotype:	Mouse IgG1, κ
Reactivity:	Human
	QC Testing: Rhesus or Baboon or Cynomolgus
Workshop:	IV A091
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

Reacts with a 28/34 kDa dimeric glycoprotein expressed early during activation of lymphocytes and monocytes. FN50 monoclonal antibody labels NK cells and most lymphocytes of the follicular mantle and perfollicular/interfollicular zone as well as intragerminal center T cells of lymph nodes and tonsils.



Flow cytometric analysis for CD69 in stimulated Rhesus macaque peripheral blood mononuclear cells (PBMC). PBMC from Rhesus macaque were stimulated for at least 4-6 hours with 20 ng/mL PMA (Sigma-Aldrich Cat. No. P-8139) and 250 ng/mL calcium ionophore A23187 (Sigma-Aldrich Cat. No. C-9275). Cells were then stained with either a APC-H7 Mouse IgG1, κ isotype control (shaded) or with the APC-H7 Mouse Anti-Human CD69 antibody (unshaded). Histograms were derived from gated events based on light scattering characteristics for lymphocytes. Flow cytometry was performed on a BD™ LSR II flow cytometry system.

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 APC-H7 under optimum conditions, and unconjugated antibody and APC-H7 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
560167	APC-H7 Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
555899	Lysing Buffer	100 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 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. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.

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4. BD APC-H7 is a tandem conjugate and an analog of APC-Cy7 with the same spectral properties. It has decreased intensity but it is engineered for greater stability and less spillover in the APC channel and consequently offers better performance than APC-Cy7. It has an absorption maximum of approximately 650 nm. When excited by light from a red laser, the APC fluorochrome can transfer energy to the cyanine dye, which then emits at a longer wavelength. The resulting fluorescent emission maximum is approximately 767 nm. BD recommends that a 750-nm longpass filter be used along with a red-sensitive detector such as the Hamamatsu R3896 PMT. As with APC-Cy7 special filters are required when using APC-H7 in conjunction with APC.
Note: Although our APC-H7 products demonstrate higher lot-to lot consistency than other APC tandem conjugate products, and every effort is made to minimize the lot-to-lot variation in residual emission from APC, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each APC-H7 conjugate.
Note: Cy is a trademark of Amersham Biosciences Limited.
5. Although BD APC-H7 is engineered to minimize spillover to the APC channel and is more stable and less affected by light, temperature, and formaldehyde-based fixatives, compared to other APC-cyanine tandem dyes, it is still good practice to minimize as much as possible, any light, temperature and fixative exposure when working with all fluorescent conjugates.
6. Cy is a trademark of Amersham Biosciences Limited.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. 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.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
10. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.

References

Knapp W, Dorken B, Rieber EP, et al, ed. *Leucocyte Typing IV*. New York: Oxford University Press; 1989. (Biology)
Schlossman S, Boumell L, et al, ed. *Leucocyte Typing V*. New York: Oxford University Press; 1995. (Biology)