Monoclonal Anti-human/mouse α-Fetoprotein-Alexa Fluor® 488

Catalog Number: IC1368G
Lot Number: ACTF01
100 Tests

Reagents Provided

Alexa Fluor® 488-conjugated mouse monoclonal anti-human/mouse α-Fetoprotein: Supplied as 10 μg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 189502

Isotype: mouse IgG_1

Reagents Not Provided

Flow Cytometry Fixation Buffer (Catalog # FC004) or other 4% paraformaldehyde fixation buffer

Flow Cytometry Permeabilization/Wash Buffer I (1X) (Catalog # FC005) or other saponin-containing saline buffer.

Storage

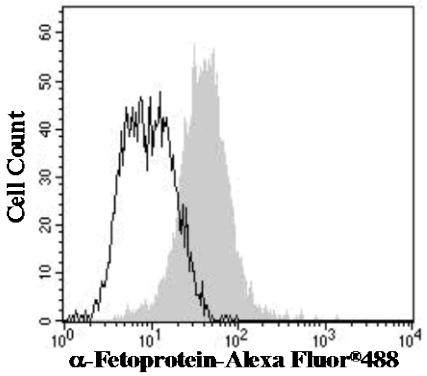
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells containing α-Fetoprotein within a population and qualitatively determine the density of intracellular α-Fetoprotein by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified human umbilical cord serum-derived α-Fetoprotein/AFP (α-Fetoprotein). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 488 fluorochrome. Intracellular expression of α-Fetoprotein is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515-545 nm.



HepG2 cells were stained with Alexa Fluor® 488-conjugated anti-human/mouse α-Fetoprotein (Catalog # IC1368G; filled histogram) or Alexa Fluor® 488-conjugated isotype control (Catalog # IC002G; open histogram).

Background Information

α-Fetoprotein is a major plasma protein in the fetus. Its concentration is normally low in the adult except when produced by certain tumors.

Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see Reagents Not Provided).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. Resuspend up to 1 x 10^6 cells in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubate at room temperature for 10 minutes.
4. Following fixation, the cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 5 μL of conjugated antibody was added and the cells were incubated for 30 minutes at room temperature **in the dark.**
6. The cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with Alexa Fluor® 488-labeled mouse IgG_1 antibody. This procedure may need to be modified, depending on the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.

Legal

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R&D Systems, Inc.
1-800-343-7475