Monoclonal Anti-human p53-APC

Catalog Number: IC13551A
Lot Number: LXZ04
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human p53: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 184727

Isotype: mouse IgG_{2B}

Reagents Not Provided

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

Flow Cytometry Staining Buffer (1x) (Catalog # FC001) or other BSA-supplemented saline buffer.

Ice-cold methanol

Storage

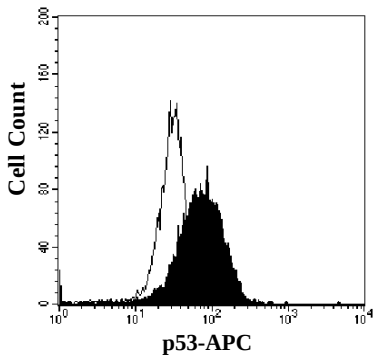
Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing p53 within a population and qualitatively determine the density of intracellular p53 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, *E. coli*-derived, recombinant human p53 (rhp53; aa 7 - 393; Accession # P04637). The IgG fraction of tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Intracellular expression of p53 is determined by flow cytometry using 620 - 650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



Intracellular staining of camptothecin-stimulated (filled histogram) or unstimulated MCF-7 cells (open histogram) with APC-conjugated anti-human p53 (Catalog # IC13551A).

Background Information

The p53 tumor suppressor protein is a multi-functional transcription factor that regulates cellular decisions regarding proliferation, cell cycle checkpoints, and apoptosis. The importance of p53 is underscored by its mutation in over 50% of human cancers. Mice that lack one or both copies of p53 show an increased incidence of tumors, which makes the p53-deficient mouse a useful model system for studying cancer generation and progression.

Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and ice-cold methanol for permeabilization (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, cells were washed twice in saline buffer, then resuspended in ice-cold methanol and incubated at 4° C for 30 minutes.
5. Cells were washed twice in Flow Cytometry Staining Buffer, then 10 μL of conjugated antibody was added and cells were incubated for 30 minutes at room temperature **in the dark**.
6. Cells were washed twice with Flow Cytometry Staining Buffer.
7. The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for analysis, cells in a separate tube should be treated with APC-labeled mouse IgG_{2B} antibody. This procedure may need to be modified, depending on cell type and final utilization. Individual users may need to titrate to determine 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.

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