Technical Data Sheet

# Alexa Fluor® 647 Mouse anti-p53 (acK382)

## Product Information

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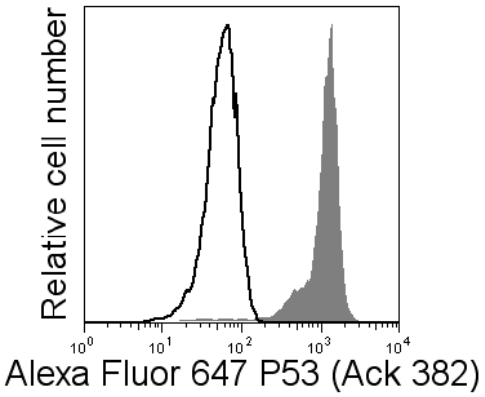
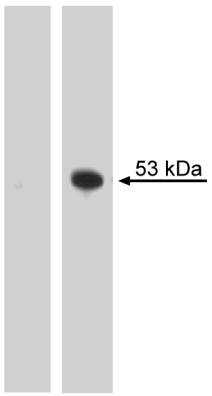
|---|---|
| **Material Number:** | **560231** |
| **Alternate Name:** | TP53 (acK382) |
| **Size:** | 50 tests |
| **Vol. per Test:** | 20 µl |
| **Clone:** | L82-51 |
| **Immunogen:** | Human p53 acetylated Peptide |
| **Isotype:** | Mouse (BALB/c) IgG1, κ |
| **Reactivity:** | QC Testing: Human |
| **Storage Buffer:** | Aqueous buffered solution containing BSA and ≤0.09% sodium azide. |

## Description

The p53 protein is critical to regulation of normal cell growth and proliferation and is a suppressor of tumor cell proliferation. Inactivation of p53 by a number of mechanisms, such as missense mutations or interaction with oncogenic viral or cellular proteins, can result in tumor progression. Mutations and/or allelic loss of the p53 gene are associated with a wide variety of human tumors. Known to have a role in transcriptional regulation, p53 suppresses various promoters containing TATA elements in an apparently sequence-independent fashion. p53 also binds to DNA in a sequence-specific manner via recognition of a 20-bp consensus-binding site. This interaction stimulates the expression of genes downstream of the p53 binding site. A number of genes that contain p53-binding sites have been identified, including MDM2, GADD45, and muscle creatine kinase. Post-translational acetylation of p53 enhances its DNA-binding activity. There are multiple factors that affect p53 acetylation, thereby modulating cellular proliferation and apoptosis.

The L82-51 monoclonal antibody recognizes acetylated lysine 382 (acK382) in the C-terminal region of p53.




***Analysis of p53 (acK382) in lymphocytes.*** *Human peripheral blood mononuclear cells (PBMC) were either treated with 0.4 µM Trichostatin A (Sigma, Cat. No. T8552) plus 0.4 µM Adriamycin (Doxorubicin hydrochloride, Sigma, Cat. No. D1515) for 24 hours (shaded histogram) or untreated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-p53 (acK382). Lymphocytes were selected by scatter profile. Flow cytometry was performed on a BD FACSArray™ bioanalyzer system.*

*The specificity of mAb L82-51 was confirmed by western blot analysis using unconjugated antibody on lysates from control (left blot) and Trichostatin A-plus-Adriamycin-treated (right blot) PBMC. p53 (acK382) is identified as a band of 53 kDa in the treated cells.*

## 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.

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*The purified or conjugated mAb was characterized by flow cytometry (Flow) and western blot (WB) using these model systems:*

| *Method* | *Species* | *Cells* | *Treatment* | *Fixation* | *Perm buffer* | *Result* |
|---|---|---|---|---|---|---|
| Flow | Human | U-2 OS | Trichostatin A + Adriamycin | Cytofix | Perm III | Unsatisfactory |
| | Human | HeLa | Trichostatin A + Adriamycin | Cytofix | Perm III | Up-regulation |
| | Human | PBMC | Trichostatin A + Adriamycin | Cytofix | Perm I, II, or III | Up-regulation |
| WB | Human | U-2 OS | Adriamycin | | | 53-kDa band induced |
| | Human | HeLa | Trichostatin A + Adriamycin | | | 53-kDa band induced |
| | Human | PBMC | Trichostatin A + Adriamycin | | | 53-kDa band induced |
| | Human | PBMC | Trichostatin A + Adriamycin + non-phospho peptide | | | No blocking of 53-kDa band |
| | Human | PBMC | Trichostatin A + Adriamycin + phospho peptide | | | Blocking of 53-kDa band |

## Application Notes

**Application**

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| Intracellular staining (flow cytometry) | Routinely Tested |

**Recommended Assay Procedure:**

This antibody conjugate is suitable for intracellular staining of human peripheral blood mononuclear cells (using BD Cytofix™ Fixation Buffer or BD Phosflow™ Fix Buffer I). Any of the three BD Phosflow™ permeabilization buffers may be used.

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 554655 | Fixation Buffer | 100 ml | (none) |
| 557885 | Perm/Wash Buffer I | 125 ml | (none) |
| 558052 | Perm Buffer II | 125 ml | (none) |
| 558050 | Perm Buffer III | 125 ml | (none) |
| 557870 | Fix Buffer I | 250 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. 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.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
5. 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.
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

## References


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