Product description

Alveolar Rhabdomyosarcoma (ARMS) is an aggressive paediatric cancer and cases with fusion oncoproteins PAX7-FOXO1 and PAX3-FOXO1 have a poor prognosis. Determination of the fusion status is important for RMS diagnostics and therapies. This antibody is specific for the PAX3-FOXO1 and PAX7-FOXO1 and therefore is a valuable tool to study the oncogenesis of ARMS.

Name: Anti-PAX-FOXO1 [PaxF]

Species Reactivity: Human

Host Species: Mouse

Strain: Balb/c

Clonality: Monoclonal

Clone: PaxF

Isotype: IgG2b kappa

Target: The junction region of the PAX3-FOXO1 and the PAX7-FOXO1 fusion proteins

Molecular weight: 105 kDa

Immunogen: LH conjugated with peptide PF corresponding to the PAX3-FOXO1 translocation region aa 100-117 (TIGNGLSPQNSIRHNLSL) in Freund's adjuvant

Conjugate: N/A

Myeloma used: P3X63Ag8.653

Applications: ChIP ; IHC ; IF ; IP ; WB ; ChIP-seq

Recommended controls: Cell lysate from FP-RMS lines (e.g., RH-4, RH-28, RH-30, RMS-13)

Contributor(s)

Institute: National Human Genome Research Institute

Properties

Concentration: 1 mg/mL

Storage buffer: PBS with 0.02% azide

Purification: Purified by affinity chromatography.

Stability and storage: Product stable at -20°C when stored undiluted. Store aliquoted, avoid repeated freeze-thaw cycles.

Directions for use: It is recommended that the antibody be titrated for optimal performance in each

application.

Western Blot Recommendations:

Detection of the fusion protein using this antibody requires stringent assay conditions to minimize nonspecific binding. The following guidelines are recommended to optimize performance:

1. Buffer Stringency: Use high-salt concentrations in all assay buffers. We recommend 0.5 M NaCl (final concentration) instead of the more typical 0.15 M.
2. Antibody Dilution: Start with a high dilution of the antibody. A 1:10,000 dilution is a good initial point for optimization.
3. Protein Loading: Load 50 µg of total protein per lane. Alternatively, using a nuclear fraction may enhance detection sensitivity.
4. Incubation Time: If signal intensity is low, consider extending the primary antibody incubation to 3 days at 4°C. Avoid reducing stringency, as this may increase background.

Immunohistochemistry Recommendations:

For optimal detection of the fusion protein by IHC, we recommend the following protocol adjustments:

1. Antigen Retrieval: Use heat-mediated antigen retrieval in EDTA buffer, pH 9 (equivalent to BOND Epitope Retrieval Solution 2) for 20 minutes. This condition has consistently produced the strongest and most specific signal.
2. Antibody Dilution: A starting dilution of 1:2,000 is recommended. However, users may need to adjust this based on their specific staining platform and tissue type.
3. Background Signal: This antibody may produce faint, diffuse nonspecific staining across various tissue types. Specific positive signal is characterised by strong nuclear staining in most tumour cells of alveolar rhabdomyosarcoma.

References

- Azorsa et al. 2021. Mod Pathol. 34(4):748-757. PMID: 33299109.
- Gryder et al. 2017. Cancer Discov. 7(8):884-899. PMID: 28446439.
- Cao et al. 2010. Cancer Res. 70(16):6497-508. PMID: 20663909.
- Khan et al. 1999. Proc Natl Acad Sci U S A. 96(23):13264-9. PMID: 10557309.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Anti-PAX-FOXO1 [PaxF] monoclonal antibody, was invented at the National Human Genome Research Institute (CancerTools.org #160866).