Mouse anti-human ZAP-70 Monoclonal Antibody (Clone 2F3.2)

REFERENCES AND PRESENTATIONS[1]

- **ready-to-use (ml)**
  MAD-000353QD–3
  MAD-000353QD–7
  MAD-000353QD–12
- **MD-Stainer presentations**[2]
  MAD-000353QD-3/V
  MAD-000353QD/V
- **concentrated**
  MAD-210353Q - 1:50 recommended dilution

COMPOSITION:
Anti-human ZAP-70 mouse monoclonal antibody purified from serum and prepared in 10mM PBS, pH 7.4, with 0.2% BSA and 0.09% sodium azide
INTENDED USE IVD: Immunohistochemistry (IHC) on paraffin embedded tissues. Not tested on frozen tissues or Western-Blotting
CLONE: 2F3.2
Ig ISOTYPE: IgG2a, kappa
IMMUNOGEN: Recombinant ZAP-70 protein including residues 1-254 and encompassing SH2 domains of human ZAP-70.
SPECIES REACTIVITY: In vitro diagnostics in humans. Not tested in other species
DESCRIPTION AND APPLICATIONS: ZAP-70 is a 70kDa protein tyrosine kinase found in T-cells and natural killer cells. Control of this protein translation is via the IgVH gene. In Western blotting of whole cell lysates of normal peripheral blood mononuclear cells, the antibody labels a band corresponding to ZAP-70.
In Western blotting of whole cell lysates of CD19-positive purified leukemia cells from patients with Ig-unmutated and Ig-mutated CLL, the antibody labels a band corresponding to ZAP-70 in the Ig-unmutated CLL samples, whereas no band is observed in the Ig-mutated CLL samples. In Western blotting of cell lysates of Jurkat cells (T-lymphoblastic cell line), the antibody labels a band of 70kDa protein. In Western blotting of cell lysates of A431 cells (carcinoma cell line), no band is observed. ZAP-70 protein is expressed in leukemic cells of approximately 25% of chronic lymphocytic leukemia (CLL) cases as well. Anti-ZAP-70 expression is an excellent surrogate marker for the distinction between the g-mutated (anti-ZAP-70 negative) and Ig-unmutated (anti-ZAP-70 positive) CLL subtypes and can identify patient groups with divergent clinical courses. The anti-ZAP-70 positive Ig-unmutated CLL cases have been shown to have a poorer prognosis.

IHC POSITIVE CONTROL: Tonsil or lymph node.
VISUALIZATION: Cytoplasmic

IHC RECOMMENDED PROCEDURE:
- 4µm thick section should be taken on charged slides; dry overnight at 60ºC
- Deparaffinise, rehydrate and HIER (heat induced epitope retrieval) – boil tissue in the Pt Module using Master Diagnóstica EDTA buffer pH8[3] for 20 min at 95ºC. Upon completion rinse with 3-5 changes of distilled or deionised water followed by cooling at RT for 20 min
- Endogenous peroxidase block - Blocking for 10 minutes at room temperature using peroxidase solution (ref. MAD-021540Q-125)
- Primary antibody: incubate for 10 minutes [The antibody dilution (when concentrated) and protocol may vary depending on the specimen preparation and specific application. Optimal conditions should be determined by the individual laboratory]
- For detection use Master Polymer Plus Detection System (HRP) (DAB included; ref. MAD-000237QK)
- Counterstaining with haematoxylin and final mounting of the slide

STORAGE AND STABILITY: up to 18 months; stored at 2-8ºC. Do not freeze.

WARNINGS AND PRECAUTIONS:
1. Avoid contact of reagents with eyes and mucous membranes. If reagents come into contact with sensitive areas, wash with copious amounts of water.
2. This product is harmful if swallowed.
3. Consult local or state authorities with regard to recommended method of disposal.
4. Avoid microbial contamination of reagents.

SAFETY RECOMMENDATIONS:
This product is intended for laboratory professional use only. The product is NOT intended to be used as a drug or for domestic purposes. The current version of

[1] These references are for presentation in vials of Low Density Polyethylene (LDPE) dropper. In case the products are used in automated stainers, a special reference is assigned as follows:
- / L: Cylindrical screw-cap vials (QD-3 / L, QD-7 / L, QD-12 / L).
- / N: Polygonal screw-cap vials (QD-3 / N, QD-7 / N, QD-12 / N).
For different presentations (references / volumes) please contact the supplier.
2 For Technical specifications for MD-Stainer, please contact your distributor.

[3] Ref: MAD-004072R/D

the Safety Data Sheet for this product can be downloaded by searching the reference number at www.vitro.bio or can be requested at regulatory.md@vitro.bio.

BIBLIOGRAPHY

1. Admirand JH, Rassidakis GZ, Abruzzo LV, et al. Immunohistochemical detection of ZAP-70 in 341 cases of non-Hodgkin and Hodgkin lymphoma. *Mod Pathol*; **17**: 954–961. 2004.
2. Sup SJ, Domiati-Saad R, Kelley TW, et al. ZAP-70 expression in B-cell hematologic malignancy is not limited to CLL/SLL. Am J Clin Pathol; **122**: 582–587. 2004.
3. Orchard JA, Ibbotson RE, Davis Z, et al. ZAP-70 expression and prognosis in chronic lymphocytic leukaemia. Lancet; **363**: 105–111. 2004.
4. Carreras J, Villamor N, Colomo L, et al. Immunohistochemical analysis of ZAP-70 expression in B-cell lymphoid neoplasms. J Pathol; **205**: 507–513. 2005.
5. Admirand JH, Knoblock RJ, Coombes KR, Tam C, Schlette EJ, pierda WG, Ferrajoli A, O'Brien S, Keating MJ, Luthra R, Medeiros LJ, Abruzzo LV. Immunohistochemical detection of ZAP70 in chronic lymphocytic leukemia predicts immunoglobulin heavy chain gene mutation status and time to progression. Modern Pathology **131**: 1–6. 2010.