Mouse Monoclonal Antibody (CAL2) Against All CALRETICULIN (CALR) Mutations

Technical Note

Procedure: Immunostaining performed manually

Alkaline Phosphatase (AP) based detection (with Biotin-Avidin amplification)

Catalog No.: DIA-CAL-100 (100µl)  DIA-CAL-250 (250µl)

Clone: CAL2

Isotype: Mouse IgG2a

Specificity: C-neoterminal of mutated CALR

Physical State: Lyophilized powder

Reconstitution:
- DIA-CAL-100 restore to 100µl
- DIA-CAL-250 restore to 250µl

Reconstitute with sterile distilled water by gentle shaking for 10 minutes

1:20-1:40 Dilution

I.

Formalin-fixed and EDTA-decalcified bone marrow biopsies:

Protocol for Biotin-Avidin-amplified Alkaline Phosphatase (AP) based detection

1. Maturation of paraffin sections in incubator (overnight at 39°C or 1h at 56°C)
2. Deparaffinization
3. Ag retrieval: 10’ cooking in pressure cooker in citrate buffer (10.5g / 5l), pH 6.0
   (it may be longer for acid decalcified tissue)
4. Cool by rinsing with water
5. Wash with distilled water and subsequently with wash buffer
6. Incubation with primary Ab at RT for 30’- 45’ (manual staining 30’ / automatic stainer ca.45’)
7. Wash with wash buffer
8. Incubate with biotinylated secondary Ab at RT for 15’
9. Wash with wash buffer
10. Incubate with Streptavidin Alkaline Phosphatase at RT for 15’
11. Wash with wash buffer
12. Incubate with substrate (Substrate buffer + chromogens + Levamisole) at RT for 20’
13. Wash with wash buffer
14. Hematoxylin staining
15. Wash with Isopropanol x2
16. Wash with Xylol x2
17. Cover with cover glass

II.

B5-fixed and EDTA-decalcified bone marrow biopsies:

Perform Ag retrieval with EDTA-buffer pH8.0, 10min pressure cooker or 30-60min microwave at 98-100°C

III.

B5-fixed and acid-decalcified bone marrow biopsies:

Perform Ag retrieval with EDTA-buffer pH8.0, 10min pressure cooker or EDTA-TRIS-buffer pH9.0, 60min microwave at 98-100°C

Results

Formalin-fixed BM: Simple and fast analysis by a specific and intense staining of megakaryocytes and a variable amount of cells with small nucleus.

B5-fixed BM: Simple and fast analysis by a specific and intense staining of megakaryocytes. Only include megakaryocytes for analysis. Sometimes weak non-specific staining of granulopoietic or erythropoietic cells.

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