

## **Protocol for Anti-Calreticulin Mutation Specific Antibody (HS-315 111-S an HS-315 111-L) Immunohistochemistry**

### **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: Incubate sections with xylene for 30 min
3. Rehydrate sections through a series of ethanol concentration ranging from 100 % to 70 % (10 % steps) with a minimum of 1 min/concentration.
4. Transfer to wash buffer (Dako/Agilent K 8007)
5. Antigen retrieval: 10 min cooking in pressure cooker in citrate buffer (10.5g / 5l), pH 6.0  
(it may be longer for acid decalcified tissue)
6. Cool by rinsing with water
7. Wash with distilled water and subsequently with wash buffer (Dako/Agilent K 8007)
8. Incubation with primary Ab at RT for 30' - 45' (manual staining 30' / automatic stainer ca.45') in antibody diluent (Dako/Agilent S2022)
9. Wash with wash buffer (Dako/Agilent K 8007)
10. Incubate with biotinylated secondary Ab at RT for 15'
11. Wash with wash buffer (Dako/Agilent K 8007)
12. Incubate with Streptavidin Alkaline Phosphatase at RT for 15'
13. Wash with wash buffer (Dako/Agilent K 8007)
14. Incubate with substrate (Substrate buffer + chromogens + Levamisole) at RT for 20'
15. Wash with wash buffer (Dako/Agilent K 8007)
16. Hematoxylin staining
17. Wash with Isopropanol x2
18. Wash with Xylol x2
19. Cover with cover glass

### **II. B5-fixed and EDTA-decalcified bone marrow biopsies:**

Perform Ag retrieval with EDTA-buffer, pH8.0, 10min in 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.