Anti-Human IDH1 R132H
Astrocytoma, Oligodendroglioma Tumor Cell Marker
Mouse Monoclonal Antibody Clone H09

Technical Note 1

Procedure: **Automated Immunostaining**
Ventana Benchmark®XT
XT ultraView DAB

Catalog No.:	**DIA-H09** (100µg) DIA-H09-M (20µg)
Clone:	H09
Concentration:	0.2mg/ml
Isotype:	Mouse IgG2a
Specificity:	Human IDH1 R132H point mutation
Physical State:	Lyophilized powder
Reconstitution:	DIA-H09 (100µg), restore to 500µl DIA-H09-M (20µg), restore to 100µl Reconstitute with sterile destilled water by gentle shaking for 10 minutes

Summary

1. Cut sections to 4 µm (Microm HM 355 S) and dry at 80° C for 15 min.
2. Dilute anti-IDH1 R132H antibody clone H09 1:20-1:50 (antibody diluent from Ventana) and fill into a Ventana antibody dispenser.
3. The Ventana staining procedure includes pretreatment with Cell Conditioner 2 (pH 6) for 60 min (standard), followed by incubation with 1:20-1:50 diluted antibody clone H09 at 37 °C for 32 min.
4. Upon antibody incubation perform Ventana standard signal amplification, ultraWash, counter- stain-ing with one drop of Hematoxylin for 4 min and one drop of bluing reagent for 4 min.
5. For chromogenic detection use ultraView Universal DAB Detection Kit (Ventana)
6. Remove slides from stainer, wash in water with a drop of dishwashing detergent and mount.

Important note:
Diffuse astrocytoma WHO grade II may have low protein-expression.
At high dilution of the antibody single tumor cells in the infiltration zone may not be stained.

Ventana Short Protocol

1. paraffin [selected]
2. dewaxing [selected]
3. heat pretreatment [selected]
4. Cell Conditioner 2 [selected]
5. Mild CC2 [selected]
6. Standard CC2 [selected]
7. define antibody incubation temperature [selected]
8. 37°C [selected]
9. antibody [selected]
10. apply 1 drop [PREP KIT 101] (antibody), incubate for [0 h 32 min]
11. amplify [selected]
12. ultraWash [selected]
13. counterstaining [selected]
14. apply 1 drop [HEMATOXYLIN] (counterstaining), apply LCS and incubate for [4 min]
15. after-counterstaining [selected]
16. apply 1 drop [BLUING REAGENT] (after-counterstaining), apply LCS, incubate for [4 min]

Ventana Full Protocol

1. ***** select EZPrep *****
2. ***** start timed steps *****
3. ***** mixer off *****
4. heat object slide up to 75°C and incubate for 4 min
5. balance EZPrep volume
6. wash slide
7. balance EZPrep volume
8. wash slide
9. balance EZPrep volume
10. apply coverslip
11. heat slide up to 75°C and incubate for 4 min
12. wash slide
13. balance dewaxing volume
14. apply coverslip
15. turn off slide heater
16. ***** mixer on *****
17. [short 8-minute-conditining]
18. wash slide
19. apply Cell Conditioner No. 2 long
20. release of Cell Cond. and Coverslip, long
21. ***** select SSC Wash *****
22. heat slide up to 94°C and incubate for 8 min
23. [mild 36-minute-conditioning]
24. apply Cell Conditioner No. 2
25. apply Cell Cond. and Coverslip (without barcode blowoff)
26. heat slide up to 95°C and incubate for 4 min
27. apply Cell Cond. and Coverslip (without barcode blowoff)
28. apply Cell Conditioner No. 2
29. apply Cell Cond. and Coverslip (without barcode blowoff)
30. apply Cell Conditioner No. 2
31. apply Cell Cond. and Coverslip (without barcode blowoff)
32. add EZPrep CC Volume Adjust
33. apply Cell Conditioner No. 2
34. apply Cell Cond. and Coverslip (without barcode blowoff)
35. apply Cell Conditioner No. 2
36. apply Cell Cond. and Coverslip (without barcode blowoff)
37. apply Cell Conditioner No. 2
38. apply Cell Cond. and Coverslip (without barcode blowoff)
39. apply Cell Conditioner No. 2
40. apply Cell Cond. and Coverslip (without barcode blowoff)
41. [standard 60-minute-conditioning]
42. apply Cell Conditioner No. 2
43. apply Cell Cond. and Coverslip (without barcode blowoff)
44. apply Cell Conditioner No. 2
45. apply Cell Cond. and Coverslip (without barcode blowoff)
46. add EZPrep CC Volume Adjust
47. apply Cell Conditioner No. 2
48. apply Cell Cond. and Coverslip (without barcode blowoff)
49. apply Cell Conditioner No. 2
50. apply Cell Cond. and Coverslip (without barcode blowoff)
51. apply Cell Conditioner No. 2
52. apply Cell Cond. and Coverslip (without barcode blowoff)
53. turn off slide heating
54. incubate for 8 min
55. rinse with reaction buffer

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56. fine adjustment of reaction buffer volume
57. apply coverslip
58. rinse with reaction buffer
59. fine adjustment of reaction buffer volume
60. apply coverslip
61. ***** synchronise procedures *****
62. heat slide up to 37°C and incubate for 4 min
63. rinse with reaction buffer
64. fine adjustment of reaction buffer volume
65. apply 1 drop UV INHIBITOR and coverslip, and incubate for 4 min
66. rinse with reaction buffer
67. fine adjustment of reaction buffer volume
68. apply coverslip
69. heat slide up to 37°C and incubate for 4 min
70. rinse with reaction buffer
71. fine adjustment of reaction buffer volume
72. apply coverslip
73. apply 1 drop [PREP KIT 101] (antibody) and incubate for [0 h 32 min]
74. rinse with reaction buffer
75. fine adjustment of reaction buffer volume
76. apply coverslip
77. heat slide up to 37°C and incubate for 4 min
78. rinse with reaction buffer
79. fine adjustment of reaction buffer volume
80. apply 1 drop AMPLIFIER A and coverslip, incubate for 8 min
81. rinse with reaction buffer
82. fine adjustment of reaction buffer volume
83. apply 1 drop AMPLIFIER B and coverslip, incubate for 8 min
84. rinse with reaction buffer
85. add 200 µl and balance reaction buffer volume
86. apply 1 drop UV HRP UNIV MULT and coverslip, incubate for 8 min
87. rinse with reaction buffer
88. fine adjustment of reaction buffer volume
89. apply coverslip
90. rinse with reaction buffer
91. fine adjustment of reaction buffer volume
92. apply coverslip
93. rinse with reaction buffer
94. fine adjustment of reaction buffer volume
95. apply 1 drop UV DAB and 1 drop UV DAB H2O2 and LCS and incubate for 8 min
96. rinse with reaction buffer
97. fine adjustment of reaction buffer volume
98. apply 1 drop UV COPPER and coverslip, incubate for 8 min
99. rinse with reaction buffer
100. fine adjustment of reaction buffer volume
101. apply 1 drop [HEMATOXYLIN] (counterstaining) and LCS, and incubate for [4 min]
102. rinse with reaction buffer
103. fine adjustment of reaction buffer volume
104. apply coverslip
105. rinse with reaction buffer
106. fine adjustment of reaction buffer volume
107. apply 1 drop [BLUING REAGENT] (after-counterstaining) and LCS, and incubate for [4 min]
108. rinse with reaction buffer
109. apply coverslip
110. turn off slide heater
111. ***** select optional washing procedure *****
112. ***** select SSC Wash *****
113. ***** start timed steps *****
114. rinse with reaction buffer

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Mouse Monoclonal Antibody Clone H09

Technical Note 2

Procedure: **Automated Immunostaining**

DAKO EnVision™ FLEX

Catalog No.:	**DIA-H09** (100µg) DIA-H09-M (20µg)
Clone:	H09
Concentration:	0.2mg/ml
Isotype:	Mouse IgG2a
Specificity:	Human IDH1 R132H point mutation
Physical State:	Lyophilized powder
Reconstitution:	DIA-H09 (100µg), restore to 500µl DIA-H09-M (20µg), restore to 100µl Reconstitute with sterile destilled water by gentle shaking for 10 minutes

Summary

1. Dewax and rehydrate 4 µm paraffin-embedded tissue sections.
2. Perform antigen retrival using EnVision™ FLEX target retrival solution at pH10 for 20min at 95°C.
3. Cool slides and treat with EnVision™ FLEX peroxidase-blocking reagent solution for 5min.
4. Incubate sections with anti-IDH1 R132H/clone H09 primary antibody at 1:20 dilution in EnVision™ FLEX antibody diluent for 20min.
5. Complete immunostainig by EnVision™ FLEX + Mouse (LINKER) / HRP technique following manufacturer's instructions.
6. Counterstain with hematoxylin and mount.

Important note:
Diffuse astrocytoma WHO grade II may have low protein-expression.
At high dilution of the antibody single tumor cells in the infiltration zone may not be stained.

DAKO EnVision™ FLEX Protocol

1. Dewax & rehydrate sections
2. Heat pretreatment: EnVision™ FLEX target retrival solution, 95°C, 20min.
3. Cool slides
4. Rinse with buffer 2x
5. Endogenous enzyme block: EnVision™ FLEX peroxidase-blocking reagent, 5min.
6. Rinse with buffer 1x
7. Primary antibody: anti-IDH1 R132H/clone H09, 1:20 in EnVision™ FLEX antibody diluent, 20min.
8. Rinse with buffer 1x
9. Secondary Reagent: EnVision™ FLEX + Mouse (LINKER), 15min.
10. Rinse with buffer 1x
11. Labelled Polymer: EnVision™ FLEX / HRP, 20min.
12. Rinse with buffer 2x
13. Substrat-Chromogen: Substrate working Solution (mix), 10min.
14. Rinse with buffer 1x
15. Counterstain: EnVision™ FLEX Hematoxylin, 5min.
16. Rinse with buffer 1x
17. Mount

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Technical Note 3

Procedure: **Immunostaining performed manually**

HRP/DAB Polymer Detection kit

Catalog No.:	**DIA-H09** (100µg) DIA-H09-M (20µg)
Clone:	H09
Concentration:	0.2mg/ml
Isotype:	Mouse IgG2a
Specificity:	Human IDH1 R132H point mutation
Physical State:	Lyophilized powder
Reconstitution:	DIA-H09 (100µg), restore to 500µl DIA-H09-M (20µg), restore to 100µl Reconstitute with sterile destilled water by gentle shaking for 10 minutes

HRP/DAB detection - Protocol

1. Dewax and rehydrate sections: Xylol: 3x10min / EtOH: 2x100%, 2x 95%, 1x70%, 1xH2O; 3min each.
2. Perform heat induced antigen retrival (HIER) using citrate buffer at pH6 (CC2 solution, Ventana) by cooking for 60min in a steamer.
3. Cool slides for 5min.
4. Wash with 3 changes of PBS buffer, 3min incubation per step
5. Blocking endogenous peroxidases: Place slides in Peroxidase-blocking solution for 10min at RT.
6. Wash with 3 changes of PBS buffer, 3min incubation per step
7. Blocking: Place slides in PBS buffer with 5% FCS and incubate for 30min at RT.
8. Cover tissue with primary antibody anti-IDH1 R132H/clone H09:
   Dilute 1:20-1:40 in PBS with 5% FCS and incubate at 4°C over night.
9. Wash with 3 changes of PBS buffer, 3min incubation per step
10. Secondary antibody: Cover tissue with Anti-mouse/rabbit polymer HRP-label for 30min at RT
11. Wash with 3 changes of PBS buffer, 3min incubation per step
12. Prepare DAB by adding 2 drops of DAB-chromogen per 1ml DAB-substrate buffer and mix
13. Staining reaction: Cover tissue with prepared DAB chromogen solution, incubate approximately for 10min. to allow for proper brown colour development.
14. Wash slides thoroughly in H_2O
15. Counterstain with hemalaun for 2min
16. Wash slides in H_2O
17. Coverslip with mounting medium (Immunoselect, dianova)

Important note:
Diffuse astrocytoma WHO grade II may have low protein-expression.
At high dilution of the antibody single tumor cells in the infiltration zone may not be stained.

Related References

1. Van den Bent MJ et al. **Interlaboratory comparison of IDH mutation detection**. *J Neurooncol* 112:173–178, 2013
2. Preusser M et al. **IDH testing in diagnostic neuropathology: review and practical guideline** article invited by the Euro-CNS research committee. *Clinical Neuropathology,* 30(5):217-230, 2011