Novocastra™ Liquid Mouse Monoclonal Antibody WAF1 Protein

Product Code: NCL-L-WAF-1

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human WAF1 protein. (Also referred to as p21, Cip1 and sdi1 protein).
Clone	4D10
Ig Class	IgG1
Antigen Used for Immunizations	Prokaryotic recombinant fusion protein corresponding to the full length WAF1 molecule.
Hybridoma Partner	Mouse myeloma (p3-NS1-Ag4-1).
Preparation	Liquid tissue culture supernatant containing 15 mM sodium azide. Volume as indicated on vial label.
Effective on Frozen Tissue	Not evaluated.
Effective on Paraffin Wax Embedded Tissue	Yes (using the high temperature antigen unmasking technique: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:20–1:40. High temperature antigen unmasking technique. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Not recommended.
Positive Controls	Immunohistochemistry: UV irradiated skin.
Staining Pattern	Nuclear.
Storage and Stability	Store liquid antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. Prepare working dilutions on the day of use.
General Overview	The gene encoding WAF1, also termed p21, is transcriptionally regulated by the tumour suppressor protein p53. Overexpression of WAF1 is growth suppressive, possibly by inhibiting the activity of cyclin/CDK complexes. One consequence of WAF1 binding to cyclin/CDK complexes is the inhibition of Rb protein phosphorylation. Induction of WAF1 expression requires wild type p53 activity in cells undergoing p53 dependent G1 arrest or apoptosis.
General References	Göhring U-J, Bersch A, Becker M, et al.. Journal of Clinical Pathology. 54: 866–870 (2001). Schwerer M J, Sailer A, Kraft K, et al.. Journal of Clinical Pathology. 54: 871–876 (2001). Tweddle D A, Malcolm A J, Cole M, et al.. American Journal of Pathology. 158 (6): 2067–2077 (2001). Garcia J F, Piris M A, Lloret E, et al.. Histopathology. 30: 120–125 (1997). Harada N, Gansauge S, Gansauge F, et al.. British Journal of Cancer. 76 (3): 299–305 (1997). Hayashi H, Miyamoto H, Ito T, et al.. American Journal of Pathology. 151 (2): 461–470 (1997). Doglioni C, Pelosio P, Laurino L, et al.. Journal of Pathology. 179: 248–253 (1996). el-Deiry W S, Harper J W, O'Connor P M, et al.. Cancer Research. 54: 1169–1174 (1994). Noda A, Ning Y, Venable S F, et al.. Experimental Cell Research. 211: 90–98 (1994). Steinman R A, Hoffman B, Iro A, et al.. Oncogene. 9: 3389–3396 (1994). el-Deiry W S, Tokino T, Velculescu V E, et al.. Cell. 75: 817–825 (1993). Xiong Y, Hannon G J, Zhang H, et al.. Nature. 366: 701–704 (1993).

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Instructions for Use

High Temperature Antigen Unmasking Technique for Immunohistochemical Demonstration on Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections in TBS* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).
Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available).
Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).
Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

** In most applications, 10 mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).*

Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ MANUFACTURER'S INSTRUCTIONS.