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p21; Clone WA-1

Catalog Number	Format	Volume
A00125-0002	(Ready-To-Use)	2 ml
A00125-0007	(Ready-To-Use)	7 ml
A00125-0025	(Ready-To-Use)	25 ml
A00125-C	(Concentrate)	1 ml

Intended Use

For In-Vitro Diagnostic Use. This antibody is intended for the qualitative visualization of the anatomical elements listed in the Specificity section. It is intended to be used within an Immunohistochemistry (IHC) procedure on formalin-fixed paraffin-embedded (FFPE) human tissue followed by visualization by light microscopy.

Description

Titer/Working Dilution: Ready-to-Use: No further dilution required.
Concentrate: Suggested dilution is 1:25

Species: Mouse

Immunogen: Recombinant human p21 protein was used as immunogen to generate the antibody.

Clone: WA-1 (HJ21)

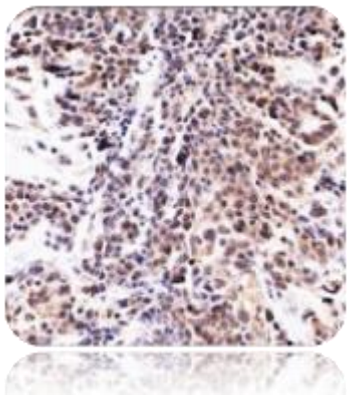
Isotype: Mouse IgG1, Kappa

Format: Ready-To-Use antibody has been pre-titered and quality controlled to work on formalin-fixed paraffin-embedded as well as acetone fixed cryostat tissue sections. No further titration is required.
Concentrate antibody is provided in a phosphate buffered saline containing 1% BSA.

Specificity: The antibody is highly specific to p21; it does not cross-react with other closely related mitotic inhibitors. Specificity validations include antibody recognition of recombinant p21 by western blot (Koike, 2011) and in direct ELISA assays (Rossner, 2002 & 2007). Although the exact epitope for the antibody has not been mapped, the epitope appears to be different from those recognize by other p21 antibody clones.

Background: p21 (WAF1), a member of the cycle dependent kinase (CDK) inhibitor family, was first identified as a tumor suppressor or anti-oncogenic protein and negative regulator of the cell cycle. Over the years it became apparent that p21 has broad functions in regulating fundamental cellular programs including proliferation, differentiation, migration, senescence, and has both anti-oncogenic and oncogenic properties (reviewed in Romanov, 2012). Many antibody and other studies have demonstrated that p21 levels are frequently elevated in situations associated with reduced proliferation, including differentiation and senescence, and decreased in proliferative states. Subcellular localization be may significant and nuclear antibody staining may be indicative of p21 functioning as a cell cycle inhibitor, tumor suppressor, or in a senescence program. In contrast, cytoplasmic antibody staining may indicate p21 is acting an oncogene through regulatation of migration, proliferation, or apoptosis.
It is interesting to note that p21 expression is induced by wild type, but not mutant p53. The HJ21/WA-1 antibody clone has been used to demonstrate this phenomenon by western blot (Blaydes, 2001). The inability of mutant p53 to induce p21 essentially means that normal functions of p21 are compromised when p53 gene mutations are present. Since p53 gene mutations are present in up to 50% of human cancers, the loss of p21 function in cancer is significant.

In its normal functioning state, p21 binds to cyclin/CDK complexes. When it binds to these complexes, it inhibits their kinase activity which, in turn, stops cell cycle progression and hence p21 gains its reputation as a mitotic cell cycle inhibitor. In this regard, the antibody showed that decreased Cdk2-cyclin E1 activity corresponded with a decrease in cyclin E1 and increase in p21 protein levels (White, 2005).



Species Reactivity: Human. Others not known.
Positive Control: Colony Carcinoma.
Cellular Localization: Primary nuclear.
Microbiological State: Nonsterile

Materials and Reagents Required but not Provided

1. Control tissue and reagents
2. Xylene, graded alcohols, and deionized/distilled water
3. Antibody Diluent.
4. IHC detection system. Suggested: ScyTek Cat# ABZ125 “CRF Anti-Polyvalent HRP Polymer” and ScyTek Cat# ACV500 “DAB Chromogen/Substrate Kit (High Contrast)”.
5. Wash buffer for rinses (ScyTek Cat# TBT500)
6. HIER Retrieval Solution
7. Hematoxylin counterstain and bluing reagent (ScyTek Cat# HMM500 and BRT500)
8. Mounting medium and coverslips

Note: ScyTek Laboratories has a wide range of IHC reagents and ancillaries that can be found at scytek.com.

Procedure

1. **Tissue Section Pretreatment (Highly Recommended):** Staining of formalin fixed paraffin embedded tissue sections is significantly enhanced by pretreatment with Tis-EDTA HIER Solution (10x) pH 9.0 (ScyTek catalog# TES500) or Citrate Plus (10x) HIER Solution (ScyTek catalog# CPL500)

2. **Primary Antibody Incubation Time:** We suggest an incubation period of 30 minutes at room temperature. However, depending upon the fixation conditions and the staining system employed, optimal incubation should be determined by the user.

3. **Visualization:** For maximum staining intensity we recommend the “CRF Anti-Polyvalent HRP Polymer” (ScyTek catalog# ABZ125, see IFU for instructions) combined

Storage: 2° C 8° C

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with the "DAB Chromogen/Substrate Bulk Pack (High Contrast)" (ScyTek catalog# ACV500, see IFU for instructions).

Storage and Stability

Do not Freeze. Store at 2-8°C. Return to 2-8° immediately after use. Do not use after expiration date printed on label. Verify visually that antibody has not been contaminated before use. Do not use if reagent becomes cloudy or precipitates.

Limitations

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining can cause inconsistent results. Variations in fixation and embedding or the inherent nature of the tissue specimen may cause variations in results. Endogenous peroxidase activity or pseudoperoxidase activity in erythrocytes and endogenous biotin may cause non-specific staining depending on detection system used. This data sheet's recommendations and procedures were validated using ScyTek IHC reagents and may not be suitable for other detection systems.

Precautions

1. Contains Sodium Azide as a preservative (0.09% w/v), do not ingest. Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. This product contains no hazardous material at a reportable concentration according to U.S. 29 CFR 1910.1200, OSHA Hazardous Communication Standard and EC Directive 91/155/EC.
2. Do not pipette by mouth.
3. Avoid contact of reagents and specimens with skin and mucous membranes.
4. Avoid microbial contamination of reagents or increased nonspecific staining may occur.
5. The user must validate any procedures and recommendations that differ from this data sheet.
6. The SDS may be found at scytek.com

References

1. Blaydes et al. JBC 276:4699-4678 (2001).
2. Scott et al. EMBO 21:6771-6780 (2002).
3. Koike et al. BBRC 412:39-43 (2011). WB (EGFP-p21 transfected NIH3T3 and TIG-1 cells), Fig 1B. Note; The specificity of the antibody was validated in EGFP-p21 transfected cells by WB.
4. Nobre et al. J Virol 83:2907-2916 (2009). Human keratinocyte oragnotypic raft cultures: IHC (paraffin).
5. Romanov et al. Biochem (Moscow) 77:575-584 (2012)
6. Rossner et al. Mutat Res 620:34-40 (2007). Direct ELISA (human blood plasma), CLONE SPECIFC Tables 1-3. Note: The specificity of the antibody was validated with recombinant p21 by direct ELISA.
7. Rossner et al. Mutat Res 517:239-250 (2007).
8. White et al. NBoC 16:2018-2027 (2005).

Warranty

No products or "Instructions For Use (IFU)" are to be construed as a recommendation for use in violation of any patents. We make no representations, warranties or assurances as to the accuracy or completeness of information provided on our IFU or website. Our warranty is limited to the actual price paid for the product. ScyTek Laboratories, Inc. is not liable for any property damage, personal injury, time or effort or economic loss caused by our products.

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