

Instructions For Use A00125-IFU-RUO

Rev. Date: Apr. 14, 2013

Revision: 1

Page 1 of 3

P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 - Tel. (435) 755-9848 - Fax (435) 755-0015 - www.scytek.com

p21; Clone WA-1 (Ready-To-Use)

Availability/Contents: <u>Item #</u> <u>Volume</u>

A00125-0002 2 ml A00125-0007 7 ml A00125-0025 25 ml

Description:

Species: Mouse

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

Clone: WA-1 (HJ21)
Isotype: Mouse IgG1, Kappa

Format: This antibody has been pretitered and quality controlled to work on formalin-fixed paraffin-

embedded as well as acetone fixed cryostat tissue sections. No further titration is required.

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 memb

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: Positive Control:

Colo

Human.

Colon Carcinoma.

Storage: 2° C 8° C

ScyTek Laboratories, Inc. 205 South 600 West Logan, UT 84321 U.S.A. CE

EmergoEurope (31)(0) 70 345-

8570

Molsnstraat 15 2513 BH Hague, The Netherlands



Instructions For Use A00125-IFU-RUO

Revision: 1

P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 - Tel. (435) 755-9848 - Fax (435) 755-0015 - www.scytek.com

Rev. Date: Apr. 14, 2013

Primarily nuclear. Cellular Localization:

Titer/Working Dilution: No further dilution is required. Microbiological State: This product is not sterile.

Uses/Limitations: Not to be taken internally.

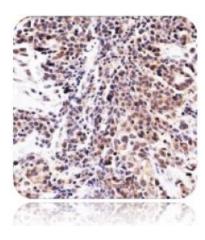
For Research Use Only.

This product is intended for qualitative immunohistochemistry with normal and neoplastic formalin-fixed, paraffin-embedded tissue sections, to be viewed by light microscopy.

Do not use if reagent becomes cloudy. Do not use past expiration date. Use caution when handling reagents.

Non-Sterile.

Ordering Information and Current Pricing at www.scytek.com



Page 2 of 3

Procedure:

- Tissue Section Pretreatment OPTIONAL: Staining of formalin fixed, paraffin embedded tissue sections is 1. enhanced by pretreatment with Citrate Plus (10X) HIER Solution (ScyTek catalog# CPL500).
- Primary Antibody Incubation Time: We suggest an incubation period of 30 minutes at room temperature. 2. However, depending upon the fixation conditions and the staining system employed, optimal incubation should be determined by the user.
- Visualization: For maximum staining intensity we recommend the "UltraTek HRP Anti-Polyvalent Lab Pack" 3. (ScyTek catalog# UHP125, see IFU for instructions) combined with the "DAB Chromogen/Substrate Bulk Pack (High Contrast)" (ScyTek catalog# ACV500, see IFU for instructions).

Precautions: Contains Sodium Azide as a preservative (0.09% w/v).

Do not pipette by mouth.

Avoid contact of reagents and specimens with skin and mucous membranes.

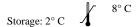
Avoid microbial contamination of reagents or increased nonspecific staining may occur.

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.

References:

- 1. Blaydes et al. JBC 276:4699-4678 (2001).
- Scott et al. EMBO 21:6771-6780 (2002). 2.
- 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.
- Nobre et al. J Virol 83:2907-2916 (2009). Human keratinocyte oragnotypic raft cultures: IHC (paraffin). 4.
- Romanov et al. Biochem (Moscow) 77:575-584 (2012)
- 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.
- Rossner et al. Mutat Res 517:239-250 (2007). 7.
- White et al. NBoC 16:2018-2027 (2005).







2513 BH Hague, The Netherlands



Instructions For Use A00125-IFU-RUO

Rev. Date: Apr. 14, 2013

Revision: 1

Page 3 of 3

P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 - Tel. (435) 755-9848 - Fax (435) 755-0015 - www.scytek.com

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. 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.

Storage: 2° C 8° C



