

Instructions For Use A00120-IFU-RUO

Rev. Date: Mar. 12, 2013

Revision: 1

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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

UchL1 (PGP9.5); Clone 31A3 (Ready-To-Use)

Availability/Contents: <u>Item #</u>
A00120-0002 <u>Volume</u>
2 ml

A00120-0002 2 ml A00120-0007 7 ml A00120-0025 25 ml

Description:

Species: Mouse

Immunogen: Native UchL1 (PGP9.5) protein from brain was used as immunogen to generate the antibody

(Day & Thompson, 1986).

Clone: 31A3

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 UchL1 clone 31A3 antibody stains neuronal cell bodies and axons in the central and

peripheral nervous systems as well as small nerve fibers in peripheral tissues, including epidermal tissues (Day & Thompson, 2010). The antibody also stains neuroendrocrine cells in the kidney, pituitary, thyroid, pancreas, gastrointestinal tract, and tumors of the diffuse neuroendocrine system. This antibody has also identified UchL1 expression in renal tubule

spermatogonia, testis, ovary, and both pregnant and non-pregnant corpus luteum.

Background:

UchL1 (ubiquitin C-terminal hydrolase), also known as PGP9.5 (protein gene product 9.5) and PARK5, is a neuronal biomarker and ubquitin system protein (reviewed in Day and Thompson, 2010). UchL1 is a highly conserved antibody and has shown that it is expressed in neurons and neuroendocrine cells in vertebrates where it comprises about 5-10% of soluble cytoplasmic proteins. A minor proportion of UchL1 in brain is tightly bound to the membrane. UchL1 antibody is also expressed in human oocytes and spermatogonia. It is important to note that the use of an antibody as a biomarker in this case does not imply that an antibody defines absolute tissue specificity. Rather, it means that PGP9.5 is expressed in neurons and neuroendrocine cells at significantly higher levels than in other cell types.

Functionally, UchL1 antibody is a thiol protease enzyme that recognizes and hydrolyzes a peptide bond at the C-terminal glycine of ubiquitin. UchL1 antibody also binds to free monoubiquitin and inhibits monoubiquitin degradation in lysosomes; it may function to stabilize monoubiquitin within neurons. A mutation in the UchL1 gene has been found to cause a form of Parkinson's disease, and this discovery has spurred considerable research interest in UchL1 and to its alternative name of PARK5.

Antibody to UchL1 is widely used as an immunohistochemical marker of nerves and neuroendocrine cells. In western blots, the antibody identifies UchL1 as a band of approximately 20-30 kDa. The antibody is specific to UchL1 and does not cross-react with the closely related UchL3 protein (Yi et al, 2007).

Storage: 2° C

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Species Reactivity: Cow, Human, Mouse, Pig, Rat. Others not tested.

Positive Control: Brain

Cellular Localization: Cytoplasmic & Cell Membrane.

Titer/Working Dilution: No further dilution is required.

Microbiological State: This product is not sterile.

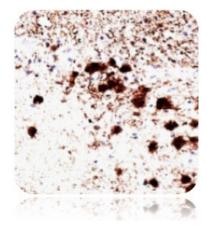
Uses/Limitations: Not to be taken internally.

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

Procedure:

- 1. **Tissue Section Pretreatment REQUIRED:** Fixation in 95% Ethanol / 5% Acetic acid for 2-3 hours prior to paraffin embedding is recommended. Staining of formalin fixed, paraffin embedded tissue sections is 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.
 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 "UltraTek HRP Anti-Polyvalent Lab Pack" (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.

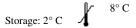
Avoid microbial contamination of reagents or increased nonspecific staining may occur.

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. Day INM and RJ Thompson. Prog Neurobiol 90:327-352 (2010). IHC (paraffin): Fig 2a,b (human brain). The distribution of PGP9.5 is extensively described in this review article, researchers are encouraged to consult this outstanding article for additional information.
- Day IN, RJ Thompson. Biochem Society Trans 14:350-351 (1986). First 31A3 antibody publication. IHC (frozen) and IHC (paraffin): various tissues; Direct ELISA: purified UchL1 protein; Protein purification (native UchL1 protein). Note: the specificity of the antibody has been validated by direct ELISA with UchL1 protein.
- 3. Sato T et al. Blood 94:2548-2554 (1999)
- 4. Bottner et al. Drew et al. Neurobiol Dis http://dx.doi.org/10.1016/j.nbd.2012.07.018 (2012). IHC (frozen); Table I (colon carcinoma, dysplastic adenoma).
- 5. Sugimoto et al. J Hyperten 29:1337-1346 (2011). IHC (frozen); Fig 3 (rat skin).









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- Gaunitz C et al. J Food Protection 72:1070-1077 (2009)). Sandwich ELISA (cow and pig meat). The antibody was used in a sandwich ELISA along with the PGP9.5 13C4 antibody clone to detect neuronal contaminant components in meat. Refer to the publication for additional details.
- 7. Stander et al. J Dermatol Sci 38:177-188 (2005). IHC (frozen); Fig 2E (human skin).
- 8. Yi et al. Biol Reprod 77:780-793 (2007). WB: Fig 6a (mouse testicular cells and spermatozoa, boar testicular cells). UchL1 was detected just between the 19 and 26 KDa markers. Note: UchL1 was not detected in boar testicular cells by WB.

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

