

**Datasheet for 600-401-Y08****AMBRA1 Antibody****Overview**

|                      |                                             |
|----------------------|---------------------------------------------|
| <b>Description:</b>  | Anti-AMBRA1 (RABBIT) Antibody - 600-401-Y08 |
| <b>Item No.:</b>     | 600-401-Y08                                 |
| <b>Size:</b>         | 100 µg                                      |
| <b>Applications:</b> | ELISA, IF, IHC, WB                          |
| <b>Reactivity:</b>   | Human, Mouse, Rat                           |
| <b>Host Species:</b> | Rabbit                                      |

**Product Details**

|                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Background:</b>   | Autophagy, the process of bulk degradation of cellular proteins through an autophagosomic-lysosomal pathway is important for normal growth control and may be defective in tumor cells. It is involved in the preservation of cellular nutrients under starvation conditions as well as the normal turnover of cytosolic components. Beclin-1, a principal regulator of autophagosome formation, is in turn regulated by Ambra1. Ambra1 associates with Beclin-1 through a region near its center as determined by yeast two-hybrid assay. Null mutations in this gene in mice resulted in embryonic lethality with severe neural tube defects associated with autophagy impairment, accumulation of ubiquitinated proteins, unbalanced cell proliferation and excessive apoptotic death. Furthermore, down-regulation of Ambra1 in cultured cells through RNA interference decreased the level of rapamycin- and nutrient starvation-induced autophagy. Multiple isoforms of Ambra1 are known to exist. |
| <b>Synonyms:</b>     | Ambra1 Antibody, DCAF3, WDR94, KIAA1736, Activating molecule in BECN1-regulated autophagy protein 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Host Species:</b> | Rabbit                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Clonality:</b>    | Polyclonal                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Format:</b>       | IgG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |

**Target Details**

|                    |                   |
|--------------------|-------------------|
| <b>Gene Name:</b>  | AMBRA1            |
| <b>Reactivity:</b> | Human, Mouse, Rat |

|                            |                                                                                                                                                                                   |
|----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Immunogen Type:</b>     | Conjugated Peptide                                                                                                                                                                |
| <b>Immunogen:</b>          | Anti-Ambra1 antibody was prepared from whole rabbit serum produced by repeated immunizations with a 15 amino acid synthetic peptide from near the C-terminus of human Ambra1.     |
| <b>Purity/Specificity:</b> | Anti-Ambra1 Antibody was affinity purified from monospecific antiserum by immunoaffinity chromatography. Cross reactivity with Ambra1 from other sources has not been determined. |
| <b>Relevant Links:</b>     | <ul style="list-style-type: none"><li>• <a href="#">UniProtKB - Q9C0C7</a></li><li>• <a href="#">GeneID - 55626</a></li><li>• <a href="#">NCBI - NP_001254711.1</a></li></ul>     |

## Application Details

|                             |                                                                                                                                                                                                                                                                                             |
|-----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Tested Applications:</b> | ELISA, IF, IHC, WB                                                                                                                                                                                                                                                                          |
| <b>Application Note:</b>    | Anti-Ambra1 Antibody has been tested for use in ELISA, Western Blotting, Immunohistochemistry and Immunofluorescence. Specific conditions for reactivity should be optimized by the end user. Expect a band at approximately 143 kDa in Western Blots of specific cell lysates and tissues. |
| <b>Assay Dilutions:</b>     | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.                                                                                                                                                                                             |
| <b>ELISA:</b>               | 1:10,000                                                                                                                                                                                                                                                                                    |
| <b>IF:</b>                  | 20 µg/mL                                                                                                                                                                                                                                                                                    |
| <b>IHC:</b>                 | 5 µg/mL                                                                                                                                                                                                                                                                                     |
| <b>WB:</b>                  | 1 µg/mL                                                                                                                                                                                                                                                                                     |

## Formulation

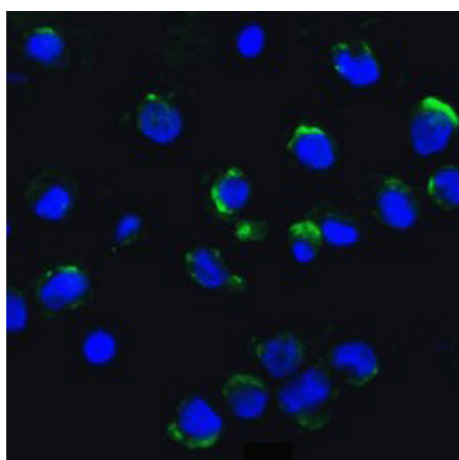
|                        |                                                         |
|------------------------|---------------------------------------------------------|
| <b>Physical State:</b> | Liquid (sterile filtered)                               |
| <b>Concentration:</b>  | 1 mg/mL by UV absorbance at 280 nm                      |
| <b>Buffer:</b>         | 0.01 M Sodium Phosphate, 0.25 M Sodium Chloride, pH 7.2 |
| <b>Preservative:</b>   | 0.02% (w/v) Sodium Azide                                |
| <b>Stabilizer:</b>     | None                                                    |

## Shipping & Handling

|                            |         |
|----------------------------|---------|
| <b>Shipping Condition:</b> | Dry Ice |
|----------------------------|---------|

|                           |                                                                                                                                                                                                                                                                                                                                               |
|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Storage Condition:</b> | Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use. |
| <b>Expiration:</b>        | Expiration date is one (1) year from date of receipt.                                                                                                                                                                                                                                                                                         |

## Images

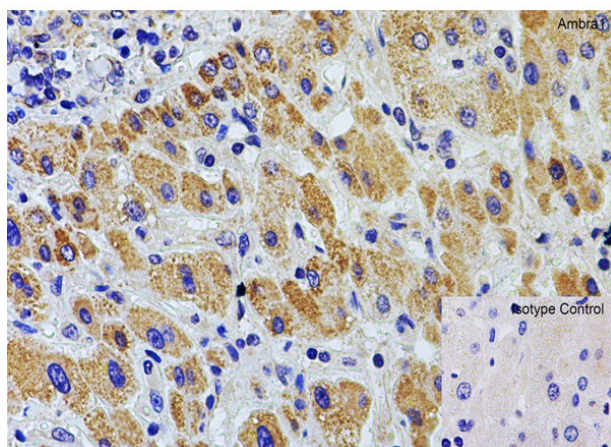


### Immunofluorescence Microscopy

Immunofluorescence of Ambra1.

Cell: K562 cells.

Primary Antibody: Ambra1 Antibody at 20 µg/ml.



### Immunohistochemistry

Immunohistochemical of Ambra1.

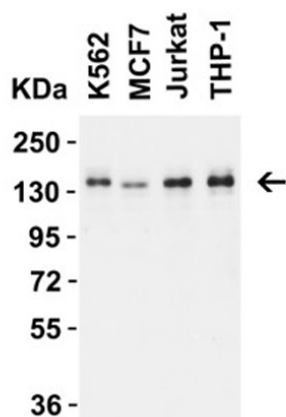
Tissue: rat colon tissue.

Fixation: paraffin-embedded, formaldehyde, and blocked with 10% serum for 1 h at RT.

Antigen retrieval: heat mediation with a citrate buffer (pH6).

Primary Antibody: anti-AMBRA1 antibody at 2 µg/ml overnight at 4°C.

Secondary: goat anti-rabbit IgG H&L (HRP) at 1:250. Counter stained with Hematoxylin.



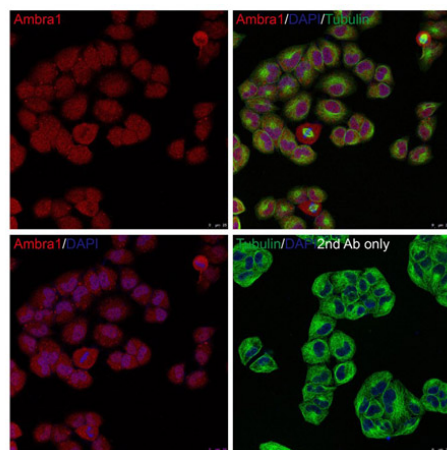
### Western Blot

Western Blot Validation of Ambra1.

Load: 15µg of human cell lysate per lane. Lane 1: K562, Lane 2: MCF7, Lane 3: Jurkat, Lane 4: THP-1.

Primary Antibody: AMBRA1 at 1 ug/mL for 1 h incubation at RT in 5% NFDN/TBST.

Secondary: Goat Anti-Rabbit IgG HRP conjugate at 1:10000 dilution.



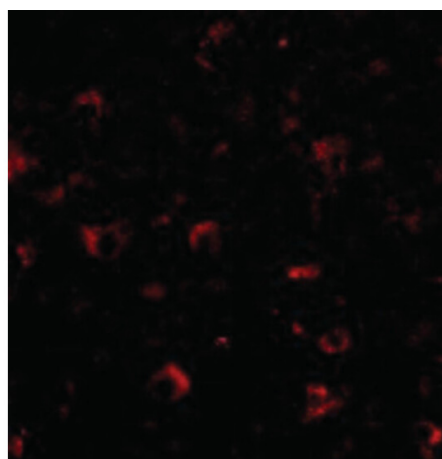
### Immunofluorescence Microscopy

Immunofluorescent of Ambra1.

Fixation: PFA-fixed HeLa cells.

Primary Antibody: AMBRA1 at 20 ug/mL.

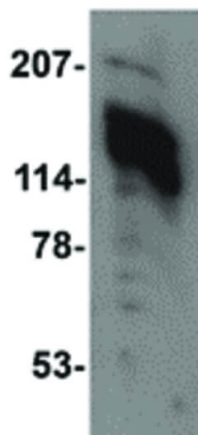
Secondary: goat anti-rabbit IgG antibody at 1:1000 dilution (red) and DAPI staining (blue). Alpha tubulin was stained with anti-alpha tubulin antibody following by goat anti-mouse IgG secondary antibody (green). Images were captured with confocal microscopy.



### Immunofluorescence Microscopy

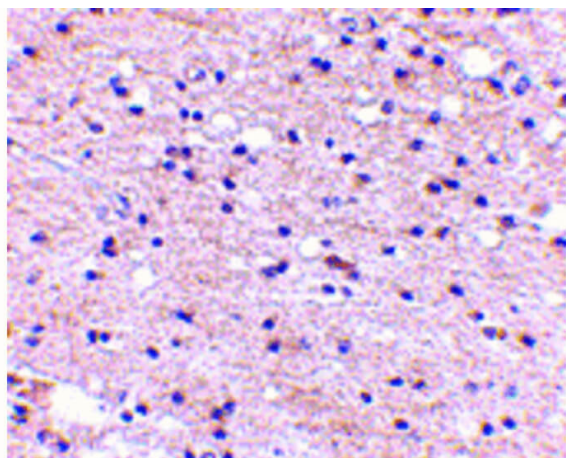
Immunofluorescence Microscopy of Ambra1 antibody.

Tissue: Human brain cells. Fixation: 0.5% PFA. Antigen retrieval: not required. Primary antibody: Ambra1 antibody at 20 µg/mL for 1 h at RT. Secondary antibody: Fluorescein rabbit secondary antibody at 1:10,000 for 45 min at RT. Staining: Ambra1 as a red fluorescent signal.



#### Western Blot

Western Blot of Ambra1 antibody. Lane 1: 3T3 cell lysate at 1 µg/mL. Load: 35 µg per lane. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 142.5 kDa, ~140 kDa for Ambra1.



#### Immunohistochemistry

Immunohistochemistry of Ambra1 antibody. Tissue: Human brain tissue. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: Ambra1 antibody at 5 µg/mL for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: Ambra1 is nuclear and occasionally cytoplasmic. Staining: Ambra1 as a precipitated red signal with hematoxylin purple nuclear counterstain.

## Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.