abcam

# ab303544

# Anti-Drosha antibody [EPR25336-93]

Recombinant | RabMAb | KO Validated | 20ul selling size

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Clone number** | EPR25336-93 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.472 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## WB

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/1000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Mouse |

| | |
|---|---|
| **Dilution info** | 1/1000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/1000 |
| **Notes** | - |

## IHC-P

### Not recommended

| | |
|---|---|
| **Species** | Human, Mouse, Rat |
| **Dilution info** | - |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/50 |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Human, Rat |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/50 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

## IP

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/30 |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | - |
| **Notes** | - |

## Target data

See full target information DROSHA

**Function**

Ribonuclease III double-stranded (ds) RNA-specific endoribonuclease that is involved in the initial step of microRNA (miRNA) biogenesis. Component of the microprocessor complex that is required to process primary miRNA transcripts (pri-miRNAs) to release precursor miRNA (pre-miRNA) in the nucleus. Within the microprocessor complex, DROSHA cleaves the 3' and 5' strands of a stem-loop in pri-miRNAs (processing center 11 bp from the dsRNA-ssRNA junction) to release hairpin-shaped pre-miRNAs that are subsequently cut by the cytoplasmic DICER to generate mature miRNAs. Involved also in pre-rRNA processing. Cleaves double-strand RNA and does not cleave single-strand RNA. Involved in the formation of

GW bodies. Plays a role in growth homeostasis in response to autophagy in motor neurons (By similarity).

## Storage

| | |
|---|---|
| **Shipped at conditions** | Conditional Ambient |
| **Appropriate short-term storage duration** | 1-2 weeks |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## Notes

**Patented technology**
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

**What are the advantages of a recombinant monoclonal antibody?**
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

We do not recommend this combination. It is not covered by our product promise.

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We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

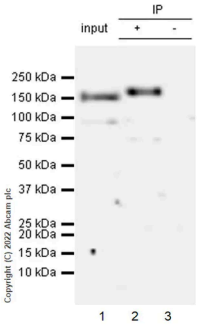
In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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## 7 product images

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### Immunoprecipitation - Anti-Drosha antibody [EPR25336-93] (ab303544)

Drosha was immunoprecipitated from 0.35 mg Hela (human cervical adenocarcinoma epithelial cell) whole cell lysate 10ug with ab303544 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab303544 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution. Lane 1: Hela (human cervical adenocarcinoma epithelial cell) whole cell lysate 10ug Lane 2: ab303544 IP in Hela whole cell lysate Lane 3:Rabbit monoclonal IgG (ab172730) instead of ab303545 in Hela whole cell lysate Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 180 seconds Lysate was freshly made and used for IP immediately to minimize protein degradation.
All lanes:
Immunoprecipitation - Anti-Drosha antibody [EPR25336-93] (ab303544) at 1/1000 dilution

All lanes:
Hela (human cervical adenocarcinoma epithelial cell) whole cell lysate

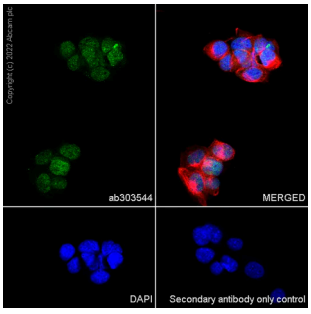
Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 180 kDa

Exposure time: 180s

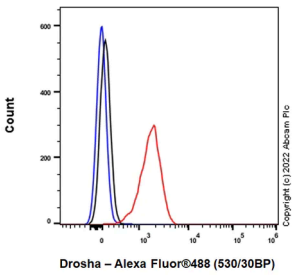
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### Immunocytochemistry/ Immunofluorescence - Anti-Drosha antibody [EPR25336-93] (ab303544)

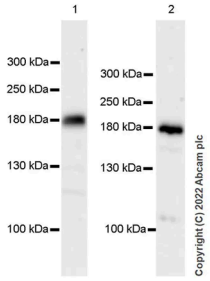
Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling Drosha with ab303544 at 1/50 (9.44 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/ml dilution (Green). Confocal image showing mainly nuclear staining in NIH/3T3 cell line. is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear

counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/ml dilution.




## Flow Cytometry - Anti-Drosha antibody [EPR25336-93] (ab303544)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling Drosha with ab303544 at 1/50 dilution (1ug) (Red) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.




## Western blot - Anti-Drosha antibody [EPR25336-93] (ab303544)

Drosha Western blot staining using rabbit Anti-Drosha antibody

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Lysates were freshly made and used for Western blotting immediately to minimize protein degradation.
All lanes:
Western blot - Anti-Drosha antibody [EPR25336-93] (ab303544) at 1/1000 dilution

Lane 1:
A549 (human lung carcinoma epithelial cell), whole cell lysate at 20 µg

Lane 2:
C2C12 (mouse myoblast), whole cell lysate at 20 µg

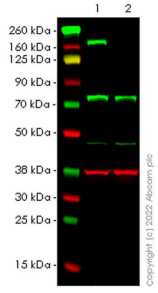
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 159 kDa

Observed band size: 180 kDa

Exposure time: 92s




## Western blot - Anti-Drosha antibody [EPR25336-93] (ab303544)

Drosha Western blot staining using rabbit Anti-Drosha antibody

Blocking and dilution buffer and concentration: ntercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS. Lysates were freshly made and used for Western blotting immediately to minimize protein degradation. The identity of the bands between 80 kDa and 40 kDa are unknown. False colour image of Western blot: Anti-Drosha antibody (ab303544) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab303544 was shown to bind specifically to Drosha. A band was observed at 180 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in Drosha knockout cell line ab266217 (knockout cell lysate ab257171). To generate this image, wild-type and Drosha knockout HEK-293T cell lysates were analyzed. First, samples were run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in in Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye®

800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
All lanes:
Western blot - Anti-Drosha antibody [EPR25336-93] (ab303544) at 1/1000 dilution

Lane 1:
Wild-type HEK-293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lanes 1 - 2:
Western blot - Human DROSHA knockout HEK-293T cell lysate (ab257171) at 20 µg

Secondary

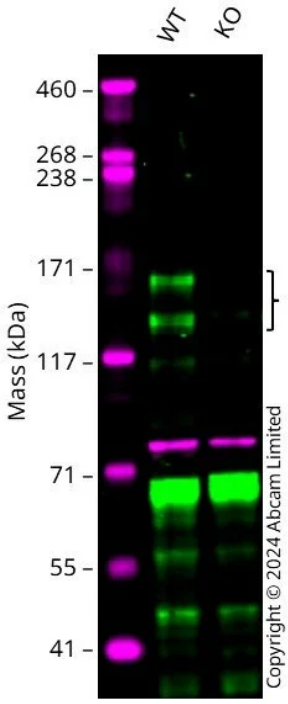
Lanes 1 - 2:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Lanes 1 - 2:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 159 kDa

Observed band size: 180 kDa




## Western blot - Anti-Drosha antibody [EPR25336-93] (ab303544)

Drosha Western blot staining using rabbit Anti-Drosha antibody

Western blot: Anti-DROSHA antibody [EPR25336-93] (ab303544) staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab303544 was shown to bind specifically to DROSHA. A band was observed at 117-171 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in DROSHA knockout cell line. To generate this image, wild-type and DROSHA knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-Drosha antibody [EPR25336-93] (ab303544) at 1/1000 dilution

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
DROSHA knockout HCT 116 cell lysate at 20 µg

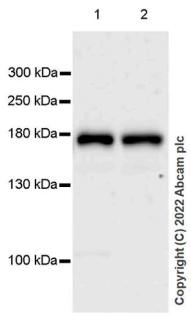
Secondary

Lanes 1 - 2:
Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution

Lanes 1 - 2:
Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 117-171 kDa




## Western blot - Anti-Drosha antibody [EPR25336-93] (ab303544)

Drosha Western blot staining using rabbit Anti-Drosha antibody

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Lysates were freshly made and used for Western blotting immediately to minimize protein degradation.
All lanes:
Western blot - Anti-Drosha antibody [EPR25336-93] (ab303544) at 1/1000 dilution

Lane 1:
NIH/3T3 (mouse embryonic fibroblast), whole cell lysate at 20 µg

Lane 2:
PC-12 (rat adrenal gland pheochromocytoma cell), whole cell lysate, at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 159 kDa

Observed band size: 180 kDa

Exposure time: 81s

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