

## Anti-HECTD3 Antibody Picoband™

Catalog Number: A11560

### About HECTD3

The protein encoded by this gene transfers ubiquitin from an E2 ubiquitin-conjugating enzyme to targeted substrates, leading to the degradation of those substrates. This gene is mapped to 1p34.1. The encoded protein has been shown to transfer ubiquitin to TRIOBP to facilitate cell cycle progression, and to STX8.

### Overview

Product Name	Anti-HECTD3 Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-HECTD3 Antibody Picoband™ catalog # A11560. Tested in Flow Cytometry, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IHC, IHC-F, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> , 0.05mg NaN <sub>3</sub> .
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q5T447

### Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human HECTD3, identical to the related mouse and rat sequences.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P), IHC(F) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.

**Suggested Dilutions**

Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.

If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.

Some PubMed article(s) citing the expression level of this target are as follows:

Boster Bio's internal QC testing used:

Western blot, 0.25-0.5 ug/ml, Mouse, Rat

Direct ELISA, 0.1-0.5 ug/ml, Human

## Anti-HECTD3 Antibody Picoband™ (A11560) Images

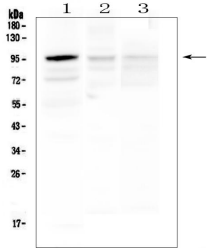


Figure 1. Western blot analysis of HECTD3 using anti-HECTD3 antibody (A11560).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human Caco-2 whole cell lysates,

Lane 2: rat brain tissue lysates,

Lane 3: mouse brain tissue lysates.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-HECTD3 antigen affinity purified polyclonal antibody (Catalog # A11560) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for HECTD3 at approximately 97KD. The expected band size for HECTD3 is at 97KD.

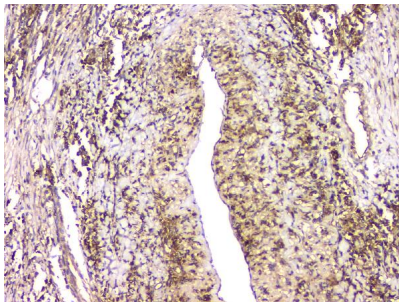


Figure 2. IHC analysis of HECTD3 using anti-HECTD3 antibody (A11560).

HECTD3 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-HECTD3 Antibody (A11560) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

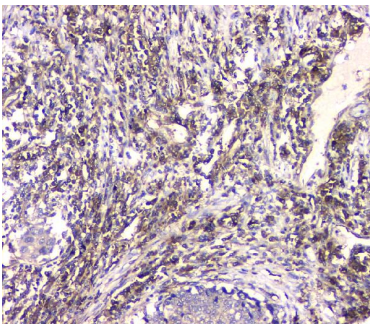


Figure 3. IHC analysis of HECTD3 using anti-HECTD3 antibody (A11560).

HECTD3 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-HECTD3 Antibody (A11560) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

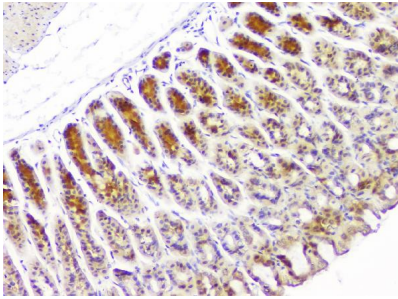


Figure 4. IHC analysis of HECTD3 using anti-HECTD3 antibody (A11560). HECTD3 was detected in paraffin-embedded section of mouse gaster tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-HECTD3 Antibody (A11560) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

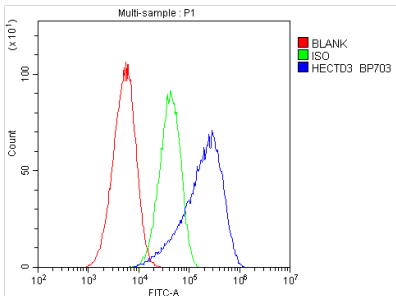


Figure 5. Flow Cytometry analysis of A549 cells using anti-HECTD3 antibody (A11560). Overlay histogram showing A549 cells stained with A11560 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-HECTD3 Antibody (A11560, 1ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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