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antibody and ELISA experts

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# Anti-TMEM16A/ANO1 Antibody Picoband™

**Catalog Number:** A00713

## About ANO1

Anoctamin-1 (ANO1), also known as oral cancer overexpressed 2 (ORAOV2) or tumor-amplified and overexpressed sequence 2 (TMEM16A), is a protein that in humans is encoded by the ANO1 gene. This gene belongs to a family of membrane proteins containing 8 transmembrane segments, and it is mapped to 11q13.3. ANO1 is a candidate calcium-activated chloride channel that mediates receptor-activated chloride currents in diverse physiologic processes, and it is thought to be responsible for a voltage-sensitive calcium-activated chloride current. Its overexpression was reported in esophageal squamous cell carcinoma and breast cancer progression Crofelemer, an antidiarrhoeal, inhibits this channel. ANO1 has eight transmembrane domains, its pore is large and non-selective, allowing other negatively charged species to permeate.

## Overview

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| Product Name | Anti-TMEM16A/ANO1 Antibody Picoband™ |
| Reactive Species | Human |
| Description | Boster Bio Anti-TMEM16A/ANO1 Antibody Picoband™ catalog # A00713. Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human. |
| Application | Flow Cytometry, IHC, WB |
| Clonality | Polyclonal |
| Formulation | Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg $Na_2HPO_4$, 0.05mg $NaN_3$. |
| 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 | Q5XXA6 |

## Technical Details

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| Immunogen | A synthetic peptide corresponding to a sequence at the C-terminus of human TMEM16A, which shares 83.8% amino acid (aa) sequence identity with both mouse and rat TMEM16A. |
| Predicted Reactive Species | Human |
| 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). |
| Cross Reactivity | No cross-reactivity with other proteins. |
| Isotype | Rabbit IgG |
| Form | Lyophilized |

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| Concentration | Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml. |
| Suggested Dilutions | Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.<br>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.<br>Some PubMed article(s) citing the expression level of this target are as follows:<br>Boster Bio's internal QC testing used:<br>Western blot, 0.1-0.5ug/ml, Human<br>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, By Heat<br>Flow Cytometry, 1-3ug/1x$10^6$ cells, Human |

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# Anti-TMEM16A/ANO1 Antibody Picoband™ (A00713) Images

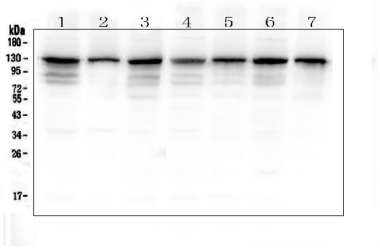


Figure 1. Western blot analysis of TMEM16A using anti-TMEM16A antibody (A00713).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysate,
Lane 2: human HepG2 whole cell lysate,
Lane 3: human A549 whole cell lysate,
Lane 4: human PANC-1 whole cell lysate,
Lane 5: human SK-OV-3 whole cell lysate,
Lane 6: human SGC-7901 whole cell lysate,
Lane 7: human COLO-320 whole cell lysate.
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-TMEM16A antigen affinity purified polyclonal antibody (Catalog # A00713) 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 TMEM16A at approximately 130KD. The expected band size for TMEM16A is at 114KD.

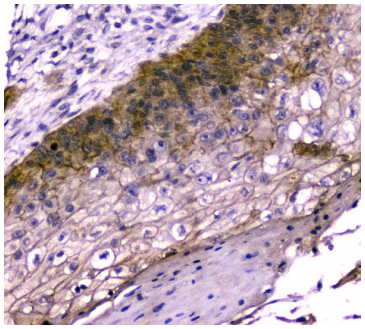

Figure 2. IHC analysis of TMEM16A using anti-TMEM16A antibody (A00713).
TMEM16A was detected in paraffin-embedded section of human oesophagus squama cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-TMEM16A Antibody (A00713) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

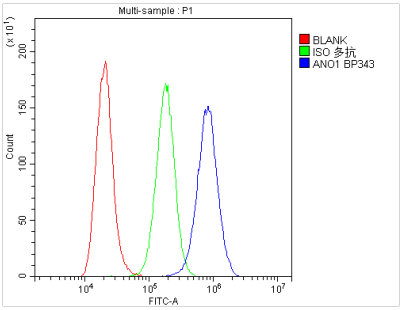


Figure 3. Flow Cytometry analysis of A431 cells using anti-TMEM16A antibody (A00713).
Overlay histogram showing A431 cells stained with A00713 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-TMEM16A Antibody (A00713,1ug/1x$10^6$ cells) for 30 min at 20°C. DyLight488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x$10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x$10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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