# **Anti-AGR2 Antibody [SN74-01]**

### ET1611-36



**Product Type:** Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human, Mouse

Applications: WB, IF-Cell, IHC-P, IP, FC

Molecular Wt: Predicted band size: 20 kDa

Clone number: SN74-01

**Description:** AGR2 (Anterior Gradient Protein 2), also known as AG2, GOB-4 or HAG-2, is a member of

the anterior gradient homolog family. It is the human ortholog of XAG-2, the secreted Xenopus laevis Anterior Gradient protein. In X. laevis, it is involved in cement gland differentiation and neural marker gene expression. AGR2 is a secretory protein encoded by two different AGR2 transcripts. It interacts with LYPD3 and  $\alpha$ -dystroglycan (DAG-1). AGR2 is ubiquitously expressed with up-regulated expression in prostate cancer, breast cancer, lung cancer, renal carcinomas and endometrial carcinomas. AGR2 expression is positively correlated with that of the estrogen receptor (ER) and is negatively correlated with that of the epidermal growth factor receptor (EGFR). AGR2 may serve as a potential therapeutic

marker for various cancers.

**Immunogen:** Synthetic peptide within Human AGR2 aa 1-50 / 175.

Positive control: NIH/3T3 cell lysate, MCF-7 cell lysate, Hela cell lysate, Hela, MCF-7, NIH/3T3, human

colon tissue.

Subcellular location: Secreted, Endoplasmic reticulum.

Database links: SwissProt: O95994 Human | O88312 Mouse

**Recommended Dilutions:** 

WB 1:1,000-1:5,000
IF-Cell 1:100-1:500
IHC-P 1:1,000
FC 1:50-1:100

IP Use at an assay dependent concentration.

**Storage Buffer:** 1\*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

**Storage Instruction:** Shipped at  $4^{\circ}$ C. Store at  $+4^{\circ}$ C short term (1-2 weeks). It is recommended to aliquot into

single-use upon delivery. Store at -20 °C long term.

**Purity:** Protein A affinity purified.

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#### **Images**

kDa ps kp s 250-150-100-72-55-45-35-25-AGR2 14-GAPDH **Fig1:** Western blot analysis of AGR2 on different lysates with Rabbit anti-AGR2 antibody (ET1611-36) at 1/1,000 dilution.

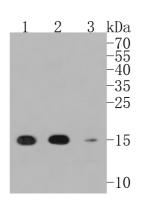
Lane 1: A549-si NT cell lysate Lane 2: A549-si AGR2 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 20 kDa Observed band size: 15 kDa

Exposure time: 30 seconds; ECL: K1801;

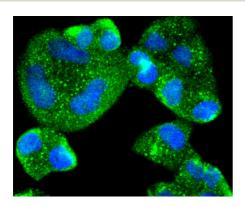
4-20% SDS-PAGE gel.



**Fig2:** Western blot analysis of AGR2 on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1611-36, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

#### Positive control:

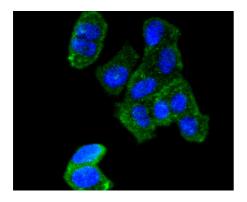
Lane 1: NIH/3T3 cell lysate Lane 2: MCF-7 cell lysate Lane 3: Hela cell lysate



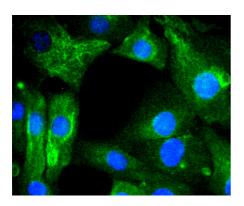
**Fig3:** ICC staining of AGR2 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-36, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

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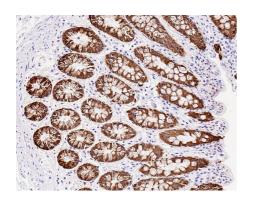
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**Fig4:** ICC staining of AGR2 in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-36, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).



**Fig5:** ICC staining of AGR2 in NIH/3T3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-36, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).



**Fig6:** Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-AGR2 antibody (ET1611-36) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1611-36) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

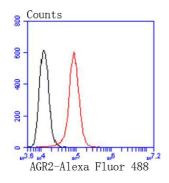


Fig7: Flow cytometric analysis of AGR2 was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1611-36, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

#### **Background References**

- 1. Li Y et al. N,N'-dinitrosopiperazine-mediated AGR2 is involved in metastasis of nasopharyngeal carcinoma. PLoS One 9:e92081 (2014).
- 2. Lee HJ et al. Identification of novel HLA-A\*0201-restricted epitopes from anterior gradient-2 as a tumor-associated antigen against colorectal cancer. Cell Mol Immunol 9:175-83 (2012).