

PRODUCT INFORMATION

Product name : ARNTL2 antibody

Product type : Primary antibodies

Description : Rabbit polyclonal to ARNTL2

Immunogen : 3 synthetic peptides (human) conjugated to KLH

Reacts with : Human, Mouse

Tested applications : ELISA, WB and IF

GENE INFORMATION

Gene Symbol : ARNTL2

Gene Name : aryl hydrocarbon receptor nuclear translocator-like 2

Ensembl ID : ENSG00000029153

Entrez GeneID : 56938

GenBank Accession number : AF246961

Swiss-Prot : Q8WYA1

Molecular weight of ARNTL2 : 70.9, 69.2, 66.5, 65.6, 65.5, 64.1 & 61.8 kDa

Function : ARNTL2-CLOCK heterodimers activate E-box element (3'-CACGTG-5') transcription. Also, in umbilical vein endothelial cells, activates SERPINE1 through E-box sites. This transactivation is inhibited by PER2 and CRY1.

Expected subcellular localization : Nucleus

Expected tissue specificity : Expressed in fetal brain. Highly expressed in brain and placenta. Lower levels in heart, liver, thymus, kidney and lung. Located to endothelial cells and neuronal cells of the suprachiasmatic nucleus (SCN). Also detected in endothelial cells of the heart, lung and kidney. In the brain, specifically expressed in the thalamus, hippocampus and amygdala.

Summary : This gene encodes a basic helix-loop-helix transcription factor belonging to the PAS (PER, ARNT, SIM) superfamily. The PAS proteins play important roles in adaptation to low atmospheric and cellular oxygen levels, exposure to certain environmental pollutants, and diurnal oscillations in light and temperature. This protein forms a transcriptionally

active heterodimer with the circadian CLOCK protein, the structurally related MOP4, and hypoxia-inducible factors, such as HIF1alpha. Consistent with its role as a biologically relevant partner of circadian and hypoxia factors, this protein is coexpressed in regions of the brain such as the thalamus, hypothalamus, and amygdala. Alternatively spliced transcript variants encoding different isoforms have been described for this gene. [provided by RefSeq, Oct 2011]

APPLICATION NOTE

Recommended dilution :

- ELISA: Antibody specificity was verified by direct ELISA against the 3 immunogen peptides. A minimum titer of 1/15000 is determined. Appropriate specificity controls were run.
- WB (recombinant protein): 1/1000.
- WB (cell lysate): 1/250.
- IF: 1/500.

Optimal dilutions/concentration should be determined by the end user.

Raised in : Rabbit

Clonality : Polyclonal

Isotype : IgG

Purity : Crude serum, Final bleed

Storage buffer : 50% glycerol containing a final concentration of 0.1% BSA and 0.01% Thimerosal.

Form : Liquid

Storage instruction : Store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

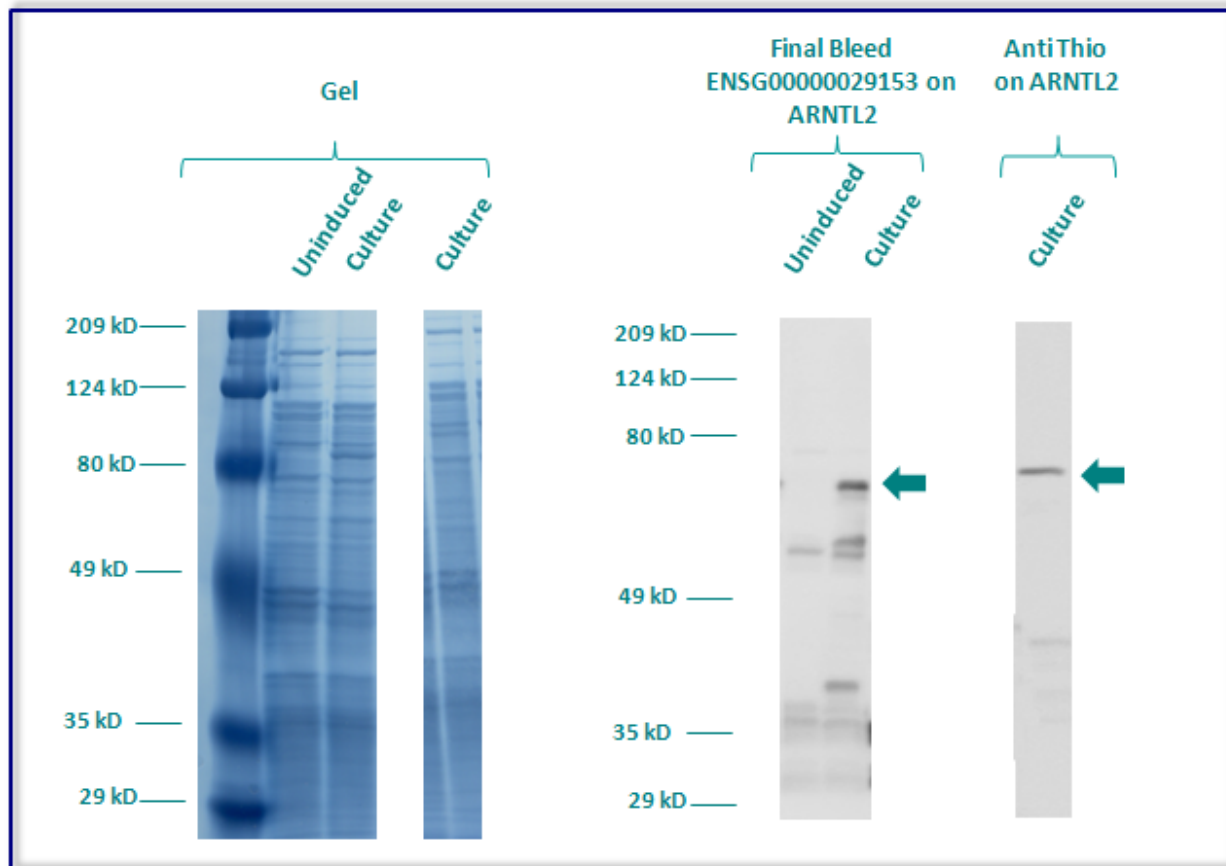
WESTERN BLOT ON RECOMBINANT PROTEIN

The final bleed ENSG00000029153 has been tested at 1/1000 on uninduced (negative control) and induced culture of E.coli (one shot Top10 competent cells).

An anti-Thio (positive control) has been tested at 1/5000 on uninduced (negative control) and induced culture of E.coli (one shot Top10 competent cells) as a positive control.

Plasmid name : pBAD-DEST49.

Molecular weight of ARNTL2 : 78.6kDa (64.6kDa + another 14kDa for the tag).



Gel concentration: 10%

Blocking: in 5% non-fat milk-PBST solution

1st Antibody: The antibodies are diluted in blocking buffer.

- Dilute the crude serum ENSG00000029153 at 1: 1000
- Dilute the anti-thio at 1:5000

60 minutes of incubation

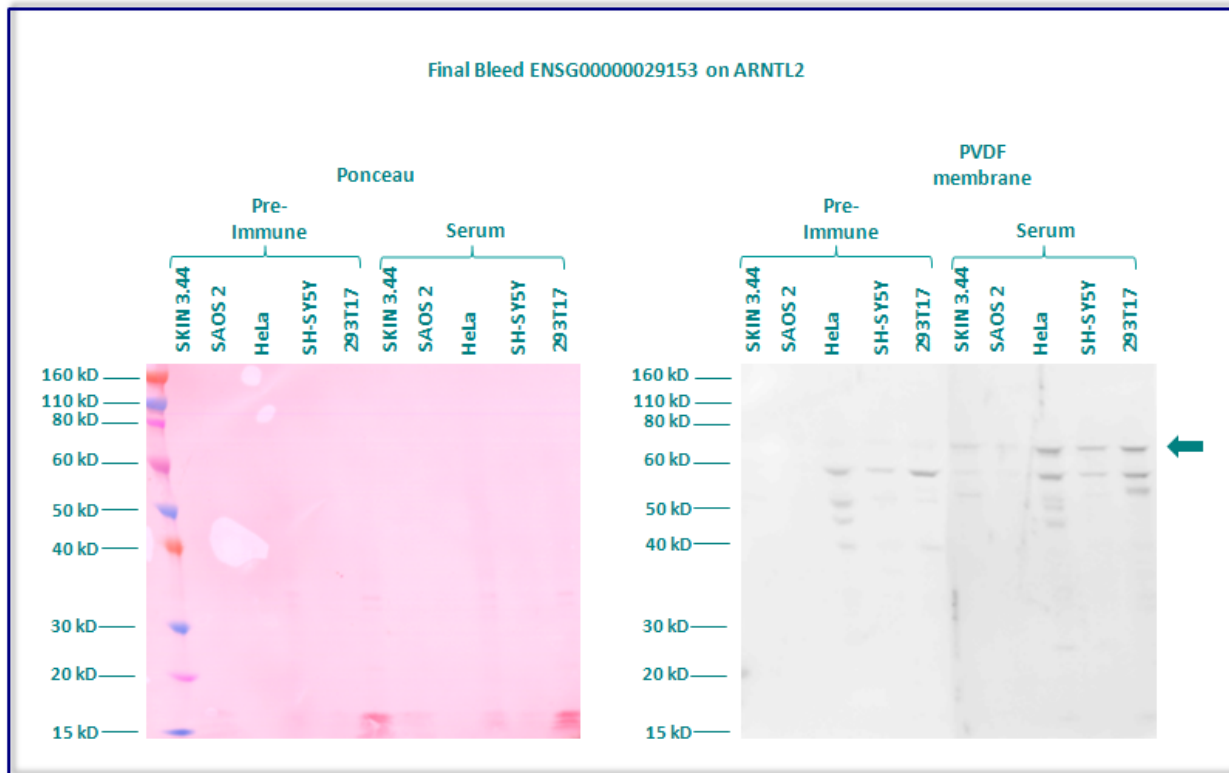
2nd Antibody: The antibody is diluted in blocking buffer.

- Dilute the anti-Rabbit IgG HRP conjugated at 1/10000
- 60 minutes of incubation

WESTERN BLOT ANALYSIS ON CELL LYSATES

The final bleed ENSG00000029153 has been tested at a concentration of 1/250 on total protein extract of various cell lines (SKIN 3.44, SAOS 2, HeLa, SH-SY5Y, & 293T17).

Molecular weight of ARNTL2 isoforms : 70.9, 69.2, 66.5, 65.6, 65.5, 65.6, 64.1 & 61.8 kDa



Gel concentration: 10%

Blocking: in 5% non-fat milk-PBST solution

1st Antibody: The antibodies are diluted in blocking buffer.

- Dilute the crude serum ENSG00000029153 at 1: 250
60 minutes of incubation

2nd Antibody: The antibody is diluted in blocking buffer.

- Dilute the anti-Rabbit IgG HRP conjugated at 1/10000
60 minutes of incubation

IMMUNOFLUORESCENCE ANALYSIS

Immunofluorescence analysis of Aryl hydrocarbon receptor nuclear translocator-like protein 2 (ARNTL2) expression in 6 cells lines (HELA, 293T/17, Capan-2, SAOS-2, SH-SY5Y, Skin 3,44). The crude serum ENSG00000029153 has been tested at 1/500.

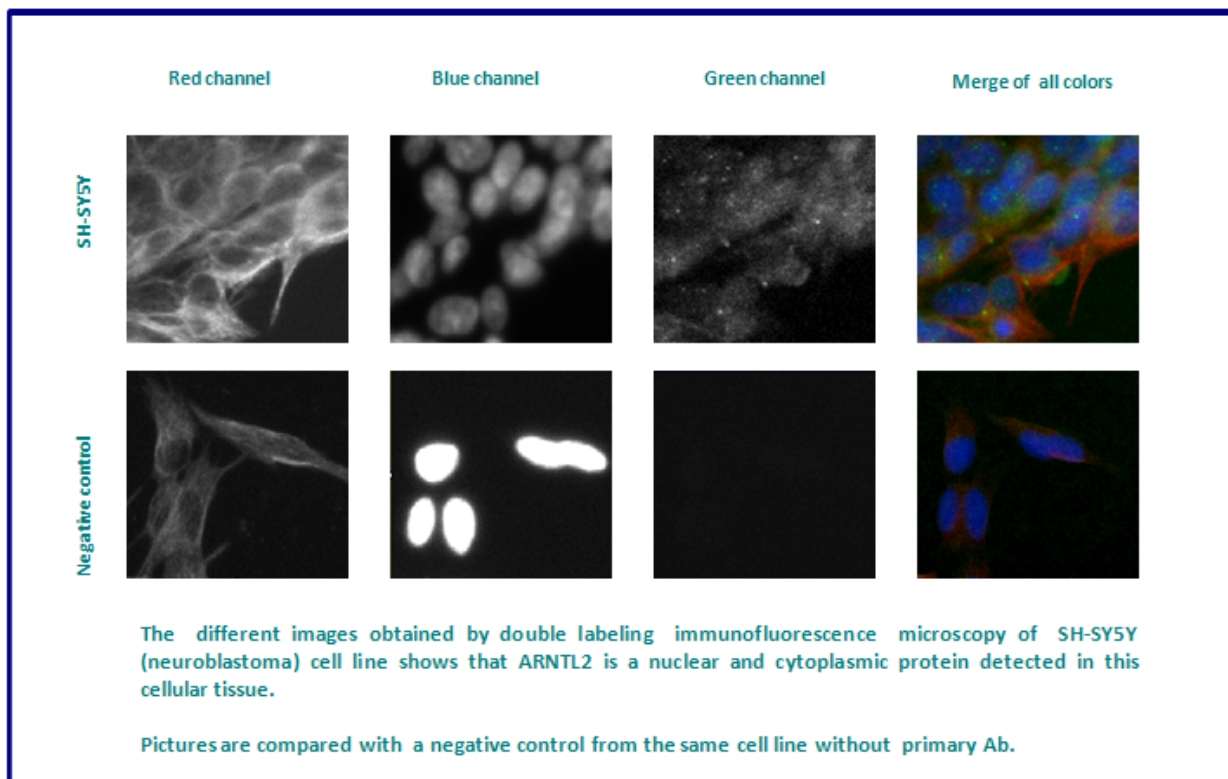
Red staining : cytoskeleton (microtubules/ α -tubuline)

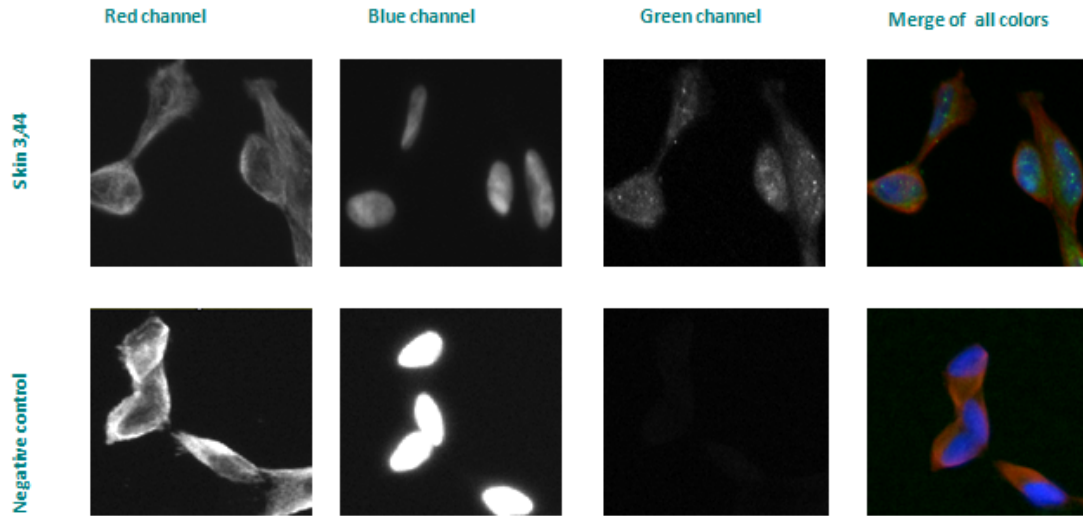
Blue staining : nucleus (Hoechst)

Green staining : anti- ARNTL2 antibody

Expected subcellular location : Nucleus

Expected tissue specificity : Expressed in fetal brain. Highly expressed in brain and placenta. Lower levels in heart, liver, thymus, kidney and lung. Located to endothelial cells and neuronal cells of the suprachiasmatic nucleus (SCN). Also detected in endothelial cells of the heart, lung and kidney. In the brain, specifically expressed in the thalamus, hippocampus and amygdale





The different images obtained by double labeling immunofluorescence microscopy of Skin 3,44 (melanoma) cell line shows that ARNTL2 is a nuclear and cytoplasmic protein detected in this cellular tissue.

Pictures are compared with a negative control from the same cell line without primary Ab.

Remaining cell lines tested gave a positive result with a nuclear and cytoplasmic distribution.