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# anti-Plakophilin 2 antibody (N-Term)

Validation

**Images** 



#### Overview

Quantity:	100 μL
Target:	Plakophilin 2 (PKP2)
Binding Specificity:	N-Term
Reactivity:	Human, Rat, Mouse, Cow, Dog, Horse
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This Plakophilin 2 antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (IHC)

#### **Product Details**

Immunogen:	The immunogen is a synthetic peptide directed towards the N terminal region of human PKP2
Sequence:	HPLRRLEISP DSSPERAHYT HSDYQYSQRS QAGHTLHHQE SRRAALLVPP
Predicted Reactivity:	Cow: 77%, Dog: 79%, Horse: 86%, Human: 100%, Mouse: 86%, Rat: 77%
Characteristics:	This is a rabbit polyclonal antibody against PKP2. It was validated on Western Blot using a cell lysate as a positive control.
Purification:	Affinity Purified

## **Target Details**

Target:	Plakophilin 2 (PKP2)
Alternative Name:	PKP2 (PKP2 Products)

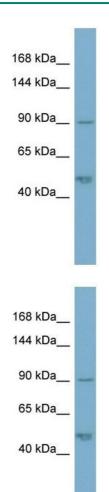
## Target Details

Background:	This gene encodes a member of the arm-repeat (armadillo) and plakophilin gene families.  Plakophilin proteins contain numerous armadillo repeats, localize to cell desmosomes and nuclei, and participate in linking cadherins to intermediate filaments in the  Alias Symbols: ARVD9  Protein Interaction Partner: NDEL1, MTUS2, CEP250, DROSHA, BMI1, EED, RNF2, YWHAB, CLK2, YWHAE, WHSC1, UBC, SMAD9, YWHAQ, CUL3, MARK3, POLR3A, YWHAG, PKP2, JUP, KRT18, KRT5, DSG2, DSG1, DSP, DSC2, DSC1, CTNNB1, GTF2B, SFN,  Protein Size: 837
Molecular Weight:	93 kDa
Gene ID:	5318
NCBI Accession:	NM_001005242, NP_001005242
UniProt:	A0AV37
Pathways:	Cell-Cell Junction Organization, SARS-CoV-2 Protein Interactome, The Global Phosphorylation Landscape of SARS-CoV-2 Infection
Application Details	
Application Notes:	Optimal working dilutions should be determined experimentally by the investigator.
Comment:	Antigen size: 837 AA
Restrictions:	For Research Use only
Handling	
Format:	Liquid
Concentration:	Lot specific
Buffer:	Liquid. Purified antibody supplied in 1x PBS buffer with 0.09 % (w/v) sodium azide and 2 % sucrose.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C

Storage Comment:

For short term use, store at 2-8°C up to 1 week. For long term storage, store at -20°C in small aliquots to prevent freeze-thaw cycles.

#### **Images**



#### **Western Blotting**

Image 1. WB Suggested Anti-PKP2 Antibody Titration: 0.2-

1 ug/ml

**ELISA Titer:** 1:312500

Positive Control: COLO205 cell lysate

#### **Western Blotting**

Image 2. WB Suggested Anti-PKP2

Antibody Titration: 0.2-1 µg/mL ELISA Titer: 1:.12500

Positive Control: COLO205 cell lysate

 $\ensuremath{\mathsf{PKP2}}$  is supported by  $\ensuremath{\mathsf{BioGPS}}$  gene expression data to be

expressed in COLO205





#### Successfully validated (Western Blotting (WB))

by Gencardio (IDIBGI)

Report Number: 103654

Date: Mar 13 2019

PKP2
QC27639-43357
Western Blotting (WB)
HL-1 cell extracts (from AT-1 mouse atrial cardiomyocyte tumor lineage)  Extracts from HEK293 cells transfected with human PKP2-FLAG expressing plasmid  Extract of mouse heart tissue (endogenous PKP2 expected molecular weight 88KDa)  Extract of human heart tissue (endogenous PKP2 expected molecular weight 91KDa)
Extracts from untransfected HEK293 cells
ABIN2786784 specifically recognizes PKP2 in cell extracts from HEK-293 overexpressing human PKP2 protein and endogenous PKP2 in human heart tissue but not endogenous mouse PKP2 neither in HL-1 cells nor mouse tissue.
ABIN2786784
mouse anti-FLAG-tag antibody (Sigma, monoclonal anti-flag, F316, lot SLBT7654)
<ul> <li>Grow HL-1 cells in Claycomb medium (Sigma, 51800C, lot SLBX8700) supplemented with 10% FBS (Gibco, 10270-106, lot 42G5178K), 1% Penicillin-Streptomycin (Sigma, P4333, lot 048M4874V) and 1% Glutamax (Gibco, 35050-061, 2037045), at 37°C and 5% CO<sub>2</sub> in 2ml on a 6 well plate to 95% confluency.</li> <li>Grow HEK293 cells in DMEM/High Modified medium (GE Life Science, SH30285.01, lot AC14565263) supplemented with 10% FBS, 1% Penicillin-Streptomycin and 1% Glutamax (Gibco, 35050-061, 2037045), at 37°C and 5% CO<sub>2</sub> in 2ml on a 6 well plate to 95% confluency.</li> <li>Transfect HEK293 cells with PKP2a plasmid (p915 PKP2a-pFLAG-cmv5a; provided by Dr. Green KJ) using Lipofectamin 2000 (Invitrogen, 11668-019, lot 2022713) following the manufacturer's instructions.</li> <li>Lyse approximately 1.2x10<sup>6</sup> cells per well in 200µl 1% SDS (Sigma, 75746, lot STBG4187V).</li> <li>Homogenate whole heart of transgenic and wt heart mouse tissue with M-Per reagent (Thermo, 78501, lot NC168900) and mechanical disgregation using a Dounce homogenizer.</li> <li>Incubate for the lysates from 15min at 95°C.</li> <li>Vortex for 15min.</li> <li>Determine total protein content of the lysates using Pierce BCA Protein Assay Kit (Thermo</li> </ul>

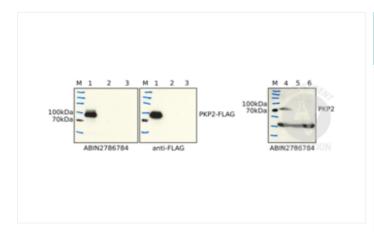
Scientific, 23227, lot TC263613).

- Denature 15μg (HEK293 cell lysates), 106μg (HL-1 cell lysates), and 100μg (mouse heart tissue lysates) of total protein for 5min at 95°C in 45µl using 6x Laemmli SDS sample buffer and subsequently separate them on a 8% acrylamide gel in an electrophoresis chamber for 30min at 80V and 1h at 120V.
- Transfer proteins onto Amersham Hybond P 0.45 PVDF blotting membrane (GE Life Science, 10600023, lot A16954274) for 2h at 80V.
- · Block the membrane with 5% NFM in PBST for 1h at RT.
- Incubation with primary
  - o rabbit anti-Plakophilin 2 (PKP2) (N-Term) antibody (antibodies-online, ABIN2786784, lot QC27639-43357) diluted 1:800 in 5% NFM in PBS ON at 4°C or
  - mouse anti-FLAG-tag antibody (Sigma, monoclonal anti-flag, F316, lot SLBT7654) diluted 1:x in 5% NFM in PBS ON at 4°C.
- Wash membrane 2x for 5' with PBST and 2x for 5min with PBS.
- Block the membrane with 5% NFM in PBST for 1h at RT.
- Incubation with secondary anti-rabbit (Jackson ImmunoResearch, 111-035-003, lot # 135002) diluted 1:10000 in 5% NFM in PBS for 1h at RT.
- Wash membrane 2x for 5min with PBST and 2x for 5min with PBS.
- · Reveal protein bands using Perce ECL Western (Thermo Scientific, 32106, TE265714) with Amersham Hyperfilm ECL (GE Healthcare Limited, 28906835, 65901).

**Experimental Notes:** 

HEK293 cells with no endogenous PKP2 expression have been transfected with human FLAGtagged PKP2 as a positive control. HL-1 cells express endogenous mouse PKP2.

#### Image for Validation report #103654



## Validation image no. 1 for anti-Plakophilin 2 (PKP2) (N-Term) antibody (ABIN2786784)

Western blot of lysates from HEK293 cells transfected with FLAG-tagged PKP2 (1), non-transfected HEK293 cells (2), non-transfected HL1 cells (3) HEK293 cells transfected with ABIN2786784 or an anti-FLAG tag antibody. In addition, lysates from human (4) and mouse heart tissue (5, 6) were incubated with ABIN2786784.