# antibodies -online.com







# anti-CITED2 antibody







#### Overview

Quantity:	50 μL
Target:	CITED2
Reactivity:	Human, Mouse, Rat
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This CITED2 antibody is un-conjugated
Application:	Western Blotting (WB)

# **Product Details**

Immunogen:	Recombinant protein of human CITED2
Isotype:	IgG
Purification:	Affinity purification

# **Target Details**

Target:	CITED2
Alternative Name:	CITED2 (CITED2 Products)
Background:	The protein encoded by this gene inhibits transactivation of HIF1A-induced genes by competing with binding of hypoxia-inducible factor 1-alpha to p300-CH1. Mutations in this gene are a cause of cardiac septal defects. Alternatively spliced transcript variants encoding multiple isoforms have been observed for this gene.
Molecular Weight:	28.497 kDa

# **Target Details**

Gene ID:	10370
UniProt:	Q99967
Pathways:	Tube Formation

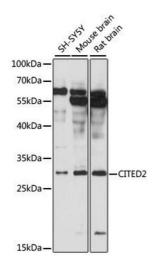
# **Application Details**

Application Notes:	WB 1:500 - 1:2000
Restrictions:	For Research Use only
Handling	
Buffer:	PBS with 0.02 % sodium azide, 50 % glycerol, pH 7.3.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C

Store at -20C. Avoid freeze / thaw cycles.

#### **Images**

Storage Comment:



#### **Western Blotting**

**Image 1.** Western blot analysis of extracts of various cell lines, using CITED2 antibody (ABIN6291914) at 1:3000 dilution. Secondary antibody: HRP Goat Anti-Rabbit IgG (H+L) at 1:10000 dilution. Lysates/proteins: 25ug per lane. Blocking buffer: 3% nonfat dry milk in TBST. Detection: ECL Enhanced Kit. Exposure time: 10s.





# Successfully validated (Western Blotting (WB))

by Dr. Pengpeng Bi Lab, Department of Genetics, Franklin College of Arts and Sciences,

University of Georgia

Report Number: 104257

Date: Feb 10 2020

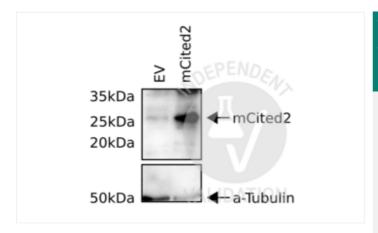
Target:	CITED2
Lot Number:	469797550101
Method validated:	Western Blotting (WB)
Positive Control:	HEK293 cells overexpressing murine Cited2 (NCBI protein ID: NP_034958, 269 amino acids) from a transiently transfected expression plasmid
Negative Control:	Transient transfection with empty vector
Notes:	Passed. ABIN6291914 specifically recognizes Cited2 in human HEK293 cell lysates.
Primary Antibody:	ABIN6291914
Secondary Antibody:	Goat anti-Rabbit IgG (H+L), Superclonal Recombinant Secondary Antibody, HRP (Invitrogen, A27036, lot 20310171)
Protocol:	<ul> <li>Grow human HEK293 cells (Cell Biolabs, RV-101) in DMEM medium(Sigma, D5796) supplemented with FBS (Gemini, A59G00J) and Pen Strep (Gibco, 15140-122, lot 1999384), at 37°C and 5% CO<sub>2</sub> in 2 mL medium on a 6 well plate to 80% confluency.</li> <li>Transfect cells with 2ug per well pMXs-Puro murine Cited2 expression plasmid using PEI (Polysciences, 23966-1) following the manufacturer's instructions.</li> <li>Lyse 1x10<sup>6</sup> cells in 100µl per well cold RIPA (Sigma, R0278).</li> <li>Determine total protein content of the lysates using Pierce BCA Protein Assay Kit(Thermo Fisher, 23225).</li> <li>Mix 40µg of total protein with in 10µL 4x Laemmli Sample Buffer (Biorad, 1610747) and subsequently separate them on a denaturing 12% PAGE gel (Biorad, 1610156) in a Biorad electrophoresis chamber for 1h at 100V.</li> <li>Transfer proteins onto Immobilon-PSQ PVDF Membrane (Sigma, ISEQ00010, lot R8CA8256B) with a Western blotting system for 1h at 110V.</li> <li>Block the membrane with blocking buffer (5% non-fat milk in TBST) for 1h at RT.</li> <li>Incubation with primary rabbit anti-CITED2 antibody (antibodies-online, ABIN6291914, lot 469797550101) diluted 1:300 in blocking buffer ON at 4°C.</li> <li>Wash membrane 3x for 15min with TBST.</li> <li>Incubation with Goat anti-Rabbit IgG (H+L), Superclonal Recombinant Secondary Antibody, HRP (Invitrogen, A27036, lot 20310171) diluted 1:2000 in blocking buffer for 1h at RT.</li> <li>Wash membrane 5x for 15min with TBST.</li> </ul>

 Reveal protein bands using SuperSignal West Dura (Thermo Scientific, 34075, TG268239) on a UVP ChemStudio touch (Analytik Jena, 84997092802).

**Experimental Notes:** 

The Cited2 antibody ABIN6291914 reveals a protein of the expected molecular weight of antigen in lysates of human HEK293 cells expressing murine Cited2. The protein band is only visible in the positive but not the negative control.

# Image for Validation report #104257



Validation image no. 1 for anti-Cbp/p300-Interacting Transactivator, with Glu/Asp-Rich Carboxy-terminal Domain, 2 (CITED2) antibody (ABIN6291914)

Western blot analysis of cell lysates from HEK293 cells transfected with an empty vector (EV) or a murine Cited2 expression plasmid (mCited2). Staining was performed using ABIN6291914 (top), or an alpha-tubulin loading control (bottom) antibody.