# antibodies - online.com







# anti-ALPK1 antibody (N-Term)







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( )	V/P	r\/I	$\Theta M$

Quantity:	100 μL
Target:	ALPK1
Binding Specificity:	N-Term
Reactivity:	Human, Mouse
Host:	Rabbit
Clonality:	Polyclonal
Application:	Western Blotting (WB), ELISA, Immunohistochemistry (IHC), Immunofluorescence (IF)

#### **Product Details**

Immunogen:	Synthesized peptide derived from the N-terminal region of human alpha-protein Kinase 1.	
Isotype:	IgG	
Specificity:	α-protein Kinase 1 Polyclonal Antibody detects endogenous levels of α-protein Kinase 1 protein.	
Characteristics:	Rabbit Polyclonal to α-protein Kinase 1.	
Purification:	The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific immunogen.	

### **Target Details**

Target:	ALPK1
Alternative Name:	alpha-Protein Kinase 1 (ALPK1 Products)
Molecular Weight:	139 kDa

# **Target Details**

Gene ID:	80216
UniProt:	Q96QP1

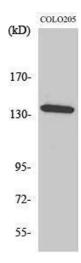
# **Application Details**

Application Notes:	WB 1:500-1:2000, IHC 1:100-1:300, IF 1:200-1:1000, ELISA 1:20000,	
Restrictions:	For Research Use only	

# Handling

Format:	Liquid
Concentration:	1 mg/mL
Buffer:	Liquid in PBS containing 50 % glycerol, 0.5 % BSA and 0.02 % sodium azide.
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:	Store at -20°C.

#### **Images**



#### **Western Blotting**

Image 1.





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

by Institute of Experimental Internal Medicine, Otto von Guericke Universität Magdeburg

Report Number: 103932

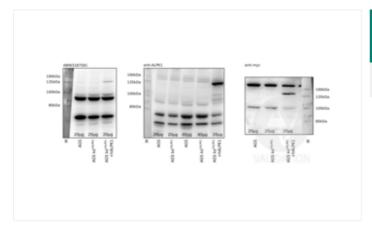
Date: Aug 26 2019

Target:	ALPK1
Lot Number:	63660
Method validated:	Western Blotting (WB)
Positive Control:	Recombinant c-Myc-tagged hALPK1 overexpression in AGS KOhALPK1
Negative Control:	AGS KO <sup>hALPK1</sup>
Notes:	ABIN3187581 detects overexpressed hALPK1 but not the endogenous protein in AGS cells.
Primary Antibody:	ABIN3187581
Secondary Antibody:	donkey anti-rabbit HRP antibody (Jackson ImmunResearch, 711-036-152, lot 140655)
Protocol:	<ul> <li>Grow AGS (ATCC, CRL-1739) in RPMI 1640 (Gibco) supplemented with 10% FCS (Gibco) and no antibiotics, at 37°C and 5% CO<sub>2</sub> in 4ml on a 60mm dish to 70% confluency.</li> <li>Transfect cells with 5µg phALPK1 (Origene) using Metafectene Pro (Biontex) following the manufacturer's instructions.</li> <li>Lyse 1.6x10<sup>6</sup> cells in 70µl per dish of ice-cold M-PER (Pierce) supplemented with Halt Protease and Phosphatase Inhibitor Cocktail (ThermoFisher, 78440) after two PBS washes.</li> <li>Determine total protein content of the lysates using BCA (ThermoFisher).</li> <li>Denature 200µg of total protein for 5min at 95°C in 100µl and subsequently separate 20µg or 40µg on a freshly cast denaturing 10% polyacrylamide gel in a Bio-Rad Protean 3 for 15min at 100V and 1.5h at 140V.</li> <li>Transfer proteins onto membrane 0.45µm PVDF (Millipore, IPVH00010) with a Bio-Rad Western blotting system for 2h at 100V constant.</li> <li>Block the membrane with TBS-T and 5% skim milk (Roth) for 1 at RT.</li> <li>Incubation with primary rabbit-anti ALPK1 antibody (antibodies-online, ABIN3187581, lot 63660) diluted 1:1000 in TBS-T containing BSA (AppliChem, A1391, 0500) ON at 4°C.</li> <li>Wash membrane 3x for 5, 10, 10 minutes with TBS-T.</li> <li>Incubation with secondary donkey anti-rabbit HRP antibody (Jackson ImmunResearch, 711-036-152, lot 140655) diluted 1:10000 in TBS-T and 5% skim milk (Roth) for 1h at RT.</li> <li>Wash membrane 3x for 10min with TBS-T.</li> <li>Reveal protein bands using Immobilon Western (Millipore, WBKLS0500, lot 1827003) using ChemoCAM (INTAS) as gel documentation system.</li> </ul>

**Experimental Notes:** 

- ABIN3187581 detected very strong two unspecific bands running at approximately 60kDa and 95kDa. This was visible incubating the antibody in milk or albumin. In neither milk nor albumin the antibody was able to detect the endogenous hALPK1 present in AGS cells. Incubation in albumin but not milk lead to the detection of recombinant overexpressed hALPK1 as confirmed by c-Myc detection (SCBT, sc-40) of the recombinant protein. Another antibody available in our laboratory (Proteintech Europe 19107-1-AP, lot 00010221) also detects the overexpressed hALPK1 (~135kDa) but not the endogenous hALPK1.
- · It is possible that the amount of ALPK1 in our cell preparation is low. Other cell lysate might be better suited. From my data, I can conclude that this antibody detects overexpressed hALPK1.
- Because no antibody detecting the endogenous hALPK1 is available to me, I cannot confirm the knockout in our cell lines, but on the functional level, we have indications for a successful knockout.

## Image for Validation report #103932



Validation image no. 1 for anti-alpha-Kinase 1 (ALPK1) (N-Term) antibody (ABIN3187581)