

## SIMPLE WESTERN CERTIFIED ANTIBODY DATASHEET

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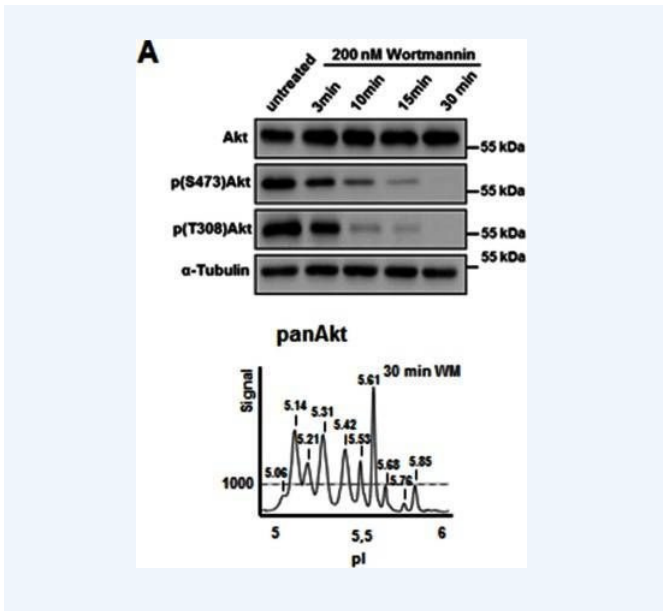


Figure 1: Dynamics of wortmannin-induced Akt dephosphorylation in N1E-115 cells. A, N1E-115 cells were treated with the PI3K inhibitor wortmannin at 200 nm for different periods of time (3–30 min) before Western blot analysis using indicated antibodies. B and C, cell lysates were analyzed in parallel using cIEF with Ser(P)473-Akt (B) and Thr(P)308-Akt (C). D and E, the area under each peak was quantified for Ser(P)473-Akt (D) and the Thr(P)308-Akt (E) signals.

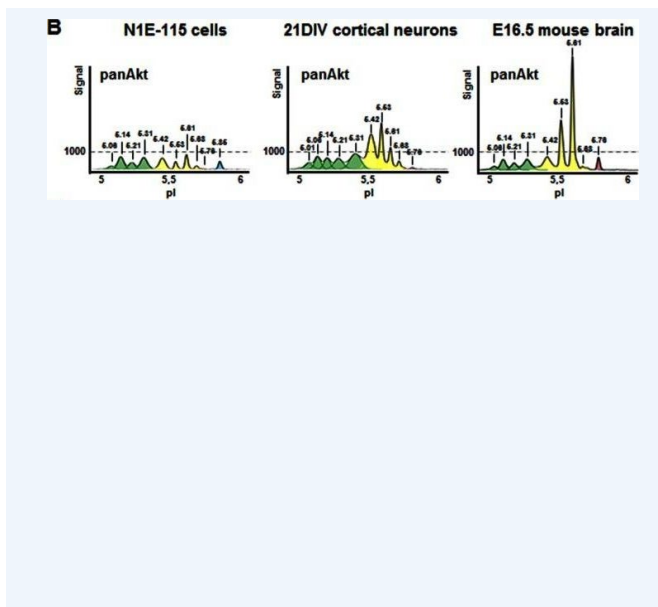


Figure 2: Akt cIEF assay development and peak identification. A, Western blot analysis of Akt isoforms (Akt1–3) in cell lysates obtained from N1E-115 neuroblastoma cells, primary cortical neurons (21DIV), and embryonic day 16.5 (E16.5) mouse brain. B, cIEF analysis of the cell lysates using a pan-Akt antibody shows a regular Akt profile with 9 or 10 conspicuous peaks that are separated according to protein charge distribution. C, cIEF analysis of cell lysates using isoform-specific Akt antibodies. In cIEF profiles, Akt1 peaks are colored in green, Akt2 peaks are in blue, and Akt3 peaks are in red. Peaks with mixed Akt isoforms are colored in yellow.

PROTEIN TARGET/ANTIBODY	
Protein Target	Akt
Protein Isoform	Unmodified
Antibody Type	Primary
Host Species/Clonality	Rabbit Polyclonal
ASSAY	
Sample Type	N1E-115, Brain
Sample Concentration	Not_Stated
Antibody Concentration/Dilution	Not_Stated
Antibody Diluent	
Detection Mode	Chemiluminescence
Separation Type	Size
Matrix	pH 5-8
Observed kDa	5.08, 5.14, 5.21, 5.31, 5.42, 5.53, 5.61, 5.63, 5.78, 5.86

PUBLICATIONS	
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3.	Goldsworthy, M., Bai, Y., et al. Haploinsufficiency of the Insulin Receptor in the Presence of a Splice-Site Mutation in Ppp2r2a Results in a Novel Digenic Mouse Model of Type 2 Diabetes. <i>Diabetes</i> . 2016 May;65(5):1434-46. 10.2337/DB15-1276. PMID:26868295.
4.	Crosbie PA, Crosbie EJ, Aspinall-O'Dea M, Walker M, Harrison R, Pernemalm M, Shah R, Joseph L, Booton R, Pierce A, Whetton AD. ERK and AKT phosphorylation status in lung cancer and emphysema using nanocapillary isoelectric focusing. <i>BMJ Open Respir Res</i> . 2
5.	Schrötter, S., Leonarditis, G., et al. Capillary Isoelectric Focusing of Akt Isoforms Identifies Highly Dynamic Phosphorylation in Neuronal Cells and Brain Tissue. <i>J Biol Chem</i> . 2016 May 6;291(19):10239-51. 10.1074/JBC.M115.700138. PMID:26945062.
6.	Padhan, N., Nordling, T. E., et al. High sensitivity isoelectric focusing to establish a signaling biomarker for the diagnosis of human colorectal cancer. <i>BMC Cancer</i> . 2016 Aug 25;16(1):683. 10.1186/S12885-016-2725-Z. PMID:27562229.
7.	Flowers, A., Bell-Temin, H., et al. Proteomic analysis of aged microglia: shifts in transcription, bioenergetics, and nutrient response. <i>J Neuroinflammation</i> . 2017 May 3;14(1):96. 10.1186/S12974-017-0840-7. PMID:28468668.
8.	Thompson, H. J., Jones, L. W., et al. Inherent aerobic capacity-dependent differences in breast carcinogenesis. <i>Carcinogenesis</i> . 2017 Sep 1;38(9):920-928. 10.1093/CARCIN/BGX066. PMID:28911004.
9.	Harun-Or-Rashid, M., Pappenhagen, N., et al. Structural and Functional Rescue of Chronic Metabolically Stressed Optic Nerves through Respiration. <i>J Neurosci</i> . 2018 May 30;38(22):5122-5139. 10.1523/JNEUROSCI.3652-17.2018. PMID:29760184.
10.	Otsuka, Y., Egawa, K., et al. Quercetin glycosides prevent dexamethasone-induced muscle atrophy in mice. <i>Biochem Biophys Rep</i> . 2019 Jul;18(NULL):100618. 10.1016/J.BBREP.2019.100618. PMID:30805562.
11.	Coulombe, P., Paliouras, G. N., et al. Endothelial Sash1 Is Required for Lung Maturation through Nitric Oxide Signaling. <i>Cell Rep</i> . 2019 May 7;27(6):1769-1780.e4. 10.1016/J.CELREP.2019.04.039. PMID:31067462.
12.	Zhu, M., Qin, Y. C., et al. Extracellular Glutamate-Induced mTORC1 Activation via the IR/IRS/PI3K/Akt Pathway Enhances the Expansion of Porcine Intestinal Stem Cells. <i>J Agric Food Chem</i> . 2019 Aug 28;67(34):9510-9521. 10.1021/ACS.JAFC.9B03626. PMID:31382738
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14.	Castillo-Castrejon, M., Jansson, T., et al. No evidence of attenuation of placental insulin-stimulated Akt phosphorylation and amino acid transport in maternal obesity and gestational diabetes mellitus. <i>Am J Physiol Endocrinol Metab</i> . 2019 Dec 1;317(6):E10
15.	Sumi, K., Ashida, K., et al. Resistance exercise with anti-inflammatory foods attenuates skeletal muscle atrophy induced by chronic inflammation. <i>J Appl Physiol (1985)</i> . 2020 Jan 1;128(1):197-211. 10.1152/JAPPLPHYSIOL.00585.2019. PMID:31804892.

## PUBLICATIONS

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17. Keleher, M. R., Erickson, K., et al. Associations between the activity of placental nutrient-sensing pathways and neonatal and postnatal metabolic health: the ECHO Healthy Start cohort. *Int J Obes (Lond).* 2020 Nov;44(11):2203-2212. 10.1038/S41366-020-0574-Y. PMID:32327723.
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