abcam

ab53025

Anti-ENO1 + ENO2 + ENO3 antibody

Lab Essentials

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.4 Preservative: 0.02% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 1% BSA, 0.88% Sodium chloride
Form	Liquid
Clonality	Polyclonal
Immunogen	Synthetic Peptide within Human ENO2. The exact immunogen used to generate this antibody is proprietary information. Database link P09104
Purification technique	Affinity purification Immunogen
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific immunogen.

Reactivity data

WB

Tested

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

Species	Human
Dilution info	1/500 - 1/1000
Notes	-

Predicted

Species	Pig, Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt

Expected

Species	Mouse
Dilution info	-
Notes	ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.

Species	Rat
Dilution info	-
Notes	ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.

Species	Pig
Dilution info	-
Notes	ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.

Species	Human
Dilution info	-
Notes	ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Recombinant fragment - Human

Dilution info	-
Notes	-

ELISA

Expected

Species	Mouse, Rat, Pig, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

IHC-FoFr

Expected

Species	Mouse, Rat, Pig, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-

Notes	-

Predicted

Species	Pig, Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1-5 µg/mL
Notes	-

Predicted

Species	Pig, Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

Target data

See full target information ENO2

Function	Enolase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate in glycolysis and the reverse reaction in gluconeogenesis (By similarity). Has neurotrophic and neuroprotective properties on a broad spectrum of central nervous system (CNS) neurons. Binds, in a calcium-dependent manner, to cultured neocortical neurons and promotes cell survival (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Storage information	Stable for 12 months at -20°C

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Neuron-specific enolase (NSE) also known simply as enolase-2 is a glycolytic enzyme with a molecular weight of approximately 47 kDa. This protein mainly facilitates the conversion of 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway. NSE is expressed significantly in neural tissues and neuroendocrine cells. Researchers often utilize it in various assays including NSE IHC and NSE ELISA to assess its expression levels in different tissue types.
Biological function summary	NSE plays a major role in cellular metabolism particularly in neurons where it aids energy production through glycolysis. It forms part of a dimeric complex bonding typically with itself or other enolase isoforms for proper function. As an important enzyme in the metabolic pathway NSE helps ensure neurons and associated cells maintain energy homeostasis which is fundamental for sustenance and normal cellular functions.
Pathways	NSE is an integral component of glycolysis a metabolic pathway pivotal in cellular energy production. The process collaborates closely with other glycolytic enzymes like pyruvate kinase and hexokinase to facilitate efficient energy release from glucose. Through these interactions NSE guarantees the smooth continuation of glycolysis highlighting its importance within cellular metabolism frameworks.
Associated diseases and disorders	NSE serves as a marker for neuroblastoma and small cell lung cancer. Elevated NSE levels suggest potential malignancies due to its release into the bloodstream from damaged neural and neuroendocrine cells. Moreover it often appears alongside proteins like neuron-specific markers such as synaptophysin in diagnostic assays. Understanding its involvement in these conditions helps clinicians diagnose and monitor the progression of certain cancers effectively.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

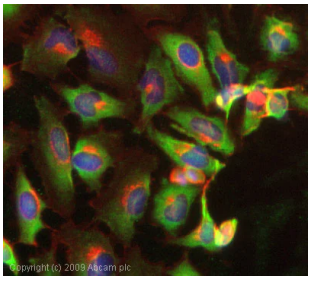
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
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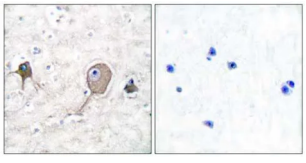
7 product images



Immunocytochemistry/ Immunofluorescence - Anti-ENO1 + ENO2 + ENO3 antibody (ab53025)

ENO1 + ENO2 + ENO3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ENO1 + ENO2 + ENO3 antibody

ICC/IF image of ab53025 stained HeLa cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab53025, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ENO1 + ENO2 + ENO3 antibody (ab53025)

ENO1 + ENO2 + ENO3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-ENO1 + ENO2 + ENO3 antibody

Immunohistochemical analysis of paraffin-embedded human brain tissue using ab53025 at 1/50-1/100 dilution. Left: without immunizing peptide; Right: with immunizing peptide

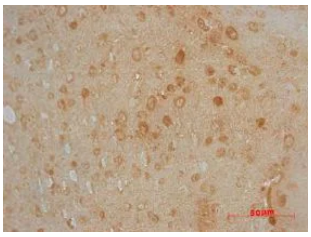
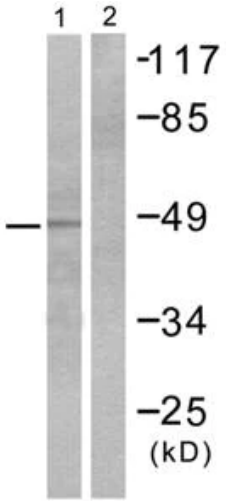


Image from Yu Yang. et. al. Mol. Cell. Proteomics, Mar 2009 (Fig 6).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ENO1 + ENO2 + ENO3 antibody (ab53025)

ENO1 + ENO2 + ENO3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-ENO1 + ENO2 + ENO3 antibody

ab53025 staining NSE in mouse brain tissue section by Immunohistochemistry (PFA-perfussion fixed frozen tissue sections). Tissues were collected from mice in each group at 10 weeks. Mice were euthanized, the brains were exposed and removed from the body. Then the brain was cut, on ice and fixed in 4% polyformaldehyde or glutaraldehyde solution for histological examination. The primary antibody was used at 1/75 dilution in PBS and incubated with sample for 2 hours at 37°C. A HRP-conjugated secondary antibody was used at for 1hr at 37°C in a humidity box.



Western blot - Anti-ENO1 + ENO2 + ENO3 antibody (ab53025)

ENO1 + ENO2 + ENO3 Western blot staining of Extracts from HepG2 cells using rabbit Anti-ENO1 + ENO2 + ENO3 antibody

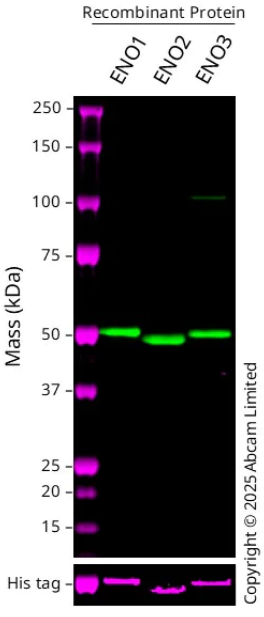
All lanes:
Western blot - Anti-ENO1 + ENO2 + ENO3 antibody (ab53025) at 1/500 dilution

Lane 1:
Extracts from HepG2 cells with no immunizing peptide

Lane 2:
Extracts from HepG2 cells with immunizing peptide

Predicted band size: 47 kDa

Observed band size: 47 kDa



Western blot - Anti-ENO1 + ENO2 + ENO3 antibody (ab53025)

ENO1 + ENO2 + ENO3 Western blot staining using rabbit Anti-ENO1 + ENO2 + ENO3 antibody

Western blot: Anti-ENO1 + ENO2 + ENO3 antibody ab53025 staining at 1/1000 dilution, shown in green; Mouse anti-6x HisTag (ab18184) loading control staining at 1/20,000 dilution, shown in magenta.

To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.

All lanes:
Western blot - Anti-ENO1 + ENO2 + ENO3 antibody (ab53025) at 1/1000 dilution

Lane 1:

ENO1 Recombinant Protein (His Tag) at 0.2 µg

Lane 2:
ENO2 Recombinant Protein (His Tag) at 0.2 µg

Lane 3:
ENO3 Recombinant Protein (His Tag) at 0.2 µg

Secondary

All lanes:
Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 47 kDa

Observed band size: 47 kDa

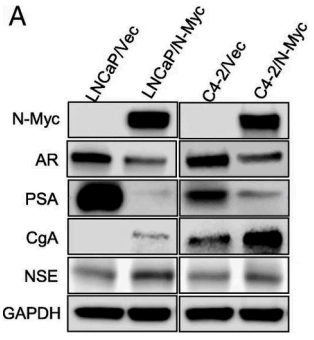


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Western blot - Anti-ENO1 + ENO2 + ENO3 antibody (ab53025)

ENO1 + ENO2 + ENO3 Western blot staining using rabbit Anti-ENO1 + ENO2 + ENO3 antibody

Western Blotting using Anti-NSE antibody - Neuronal Marker, ab53025. Publication image from Yin, Y. et al., 2019, Mol Cancer, 30657058. Legend direct from paper.

N-Myc confers LNCaP cells ADT resistance and C4–2 cells Enzalutamide resistance. a Immunoblots to show the decreased AR and PSA expression but increased expression of neuroendocrine markers, CgA and NSE, in both N-Myc overexpressing LNCaP and C4–2 cells. GAPDH was used as a loading control. b Growth curve for LNCaP/Vec vs. LNCaP/N-Myc and C4–2/Vec vs. C4–2/N-Myc cells cultured in regular medium and cell number was counted at the indicated time points. All the numbers were normalized to day 0. c Overexpression of N-Myc increased colony formation in both LNCaP and C4–2 cell lines but had a more profound effect on LNCaP cells. Upper panels showed representative images and lower panels were quantifications. d Growth curve for LNCaP/Vec and LNCaP/N-Myc cells cultured in charcoal-stripped medium (ADT) and cell numbers was counted at the indicated time points. All the numbers were normalized to day 0. e LNCaP/Vec and LNCaP/N-Myc cells were injected into the flanks of nude mice. Tumor volume was measured twice a week at indicated time points (n = 4 for each group). f Castration was initiated when tumor volume reached ~ 200mm3 and set as day 0. Tumor volume was measured twice a week and relative volume was reported (n = 4 for each group). g Dose response of Enzalutamide at 72 h using MTS cell viability assay for C4–2/Vec and C4–2/N-Myc cells. *p < 0.05, **p < 0.005

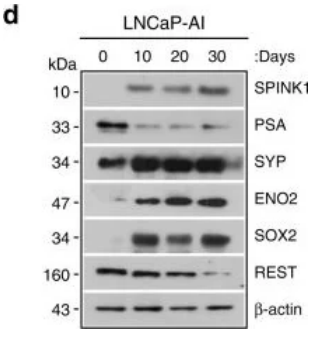


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Western blot - Anti-ENO1 + ENO2 + ENO3 antibody (ab53025)

ENO1 + ENO2 + ENO3 Western blot staining using rabbit Anti-ENO1 + ENO2 + ENO3 antibody

Western Blotting using Anti-NSE antibody - Neuronal Marker, ab53025. Publication image from Poutanen, M. et al., 2020, Nat Commun, 31959826. Legend direct from paper.

Androgen-deprivation upregulates SPINK1 in NE-transdifferentiated PCa cells.a Bar graph showing SPINK1 expression (top) and heatmap of AR-signaling associated genes including SPINK1 in long-term androgen deprived (AD) LNCaP cells (GSE8702). b Representative phase-contrast images of androgen-deprived LNCaP cells (LNCaP-AI). Red arrow-heads indicate neurite outgrowth. c QPCR data showing relative expression of SPINK1 and KLK3 using same cells as b. d Immunoblot assay for SPINK1, PSA, SYP, ENO2, SOX2 and REST using same cells as in b. e Immunostaining for SPINK1 using same cells as in b. f Schematic representation of NE-transdifferentiation (NE-td) using doxycycline (dox)-inducible AR-V7 overexpressing LNCaP cells subjected to androgen deprivation

(AD) with or without induction (40 ng/ml) at day 10 and cultured upto 30 days. g QPCR data showing relative expression of SPINK1 and KLK3 using same cells as f. h Immunoblot assay for AR, AR-V7, SPINK1, and PSA using same cells as f. i QPCR data showing relative expression of SYP, NCAM1, ENO2 and CHGA using the same cells as f. j Schema describing generation of LNCaP-AI-shSPINK1 and LNCaP-AI-shSCRM cells by subjecting stable LNCaP-shSPINK1 and LNCaP-shSCRM cells to androgen deprivation (AD) for 30 days. k Representative images for the neurite outgrowths in LNCaP-AI-shSCRM cells and LNCaP-AI-shSPINK1 as j (top). QPCR data showing relative expression of SPINK1 (bottom, left) and measurement of neurite outgrowth (bottom, right). l Immunoblot analysis for SPINK1, E-Cad, VIM, and N-Cad expression using same cells as j. m Same as in l, except phospho (p) and total (t) AKT, SYP and ENO2 expression. n Representative IHC images for SPINK1 in SPINK1-negative (SPINK1−, top) and SPINK1-positive (SPINK1+, bottom) PCa tumor cores of the VPC tissue microarray (scale bar = 200 µm). Bar plot showing percentage cases of SPINK1 in untreated (n = 33) and neoadjuvant-hormone therapy (NHT) treated patients (n = 55). Experiments were performed with n = 3 biologically independent samples; data represents mean ± SEM. For panels b, e, k scale bar represents 20 µm. For panel c two-way ANOVA, Dunnett’s multiple-comparisons; g, i two-way ANOVA, Sidak’s multiple-comparisons test; k one-way ANOVA, Dunnett’s multiple-comparisons test were applied. *P ≤ 0.05 and **P ≤ 0.001. Source data for d, h, l, m are provided as a Source Data file.

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