# PRODUCT INFORMATION



## **HNEJ-1 Monoclonal Antibody (Clone IG10)**

Item No. 38404

### **Overview and Properties**

This vial contains 100 µg of protein G-purified monoclonal antibody. Contents:

Synonyms: 4-HNE, 4-hydroxy Nonenal Immunogen: 4-HNE-modified KLH Cross Reactivity: (+) 4-HNE-modified proteins Species Reactivity: (+) Species independent

Liquid Form:

-20°C (as supplied) Storage:

Stability: ≥3 years

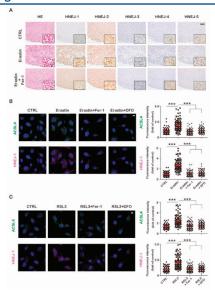
Storage Buffer: PBS, pH 7.2, with 50% glycerol and 0.02% sodium azide

Clone: IG10 Host: Mouse Isotype: lgG1

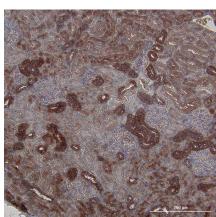
**Applications:** Immunofluorescence (IF), Immunohistochemistry (IHC), Immunoprecipitation (IP), and

Western blot (WB) applications; the recommended starting dilution for IF and IHC is 1:100-200, 5-10 µg per IP test, and 1:500 for WB. Other applications were not tested, therefore optimal working concentration/dilution should be determined empirically.

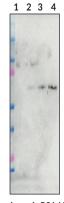
#### **Images**



Identification of HNEJ-1 as a ferroptosis-specific antibody among five monoclonal anti-HNE antibodies. (A) Immunohistochemical staining of paraformaldehyde-fixed HT-1080 human fibrosarcoma cells using HNEJ1-5 antibodies (scale bar =  $100 \mu m$ ). Cells were incubated with 10  $\mu$ M erastin in the presence or absence of 5  $\mu$ M ferrostatin-1 (Fer-1) for 12 h. (B) Immunofluorescent labeling of paraformaldehyde-fixed HT-1080 cells using HNEJ-1 or anti-ACSL4 and visualized with confocal microscopy (scale bar = 10 µm). Cells were incubated with 10  $\mu$ M erastin in the presence or absence of 5  $\mu$ M Fer-1 or 500  $\mu$ M deferoxamine mesylate (DFO) for 12 h. (C) Immunofluorescent labeling of paraformaldehyde-fixed HT-1080 cells using HNEJ-1 or anti-ACSL4 and visualized with confocal microscopy (scale bar =  $10 \mu m$ ). Cells were incubated with 0.25  $\mu M$  RSL3 in the presence or absence of 5  $\mu M$ Fer-1 or 500  $\mu$ M DFO for 3 h. Representative data are shown based on three independent experiments; \*\*\*p < 0.001 vs control (CTRL).<sup>2</sup>



Immunohistochemistry analysis of formalin-fixed paraffin-embedded (FFPE) rat kidney after heat-induced antigen retrieval in pH 6.0 citrate buffer. After incubation with a 1:100 dilution of HNEJ-1 Monoclonal Antibody (Clone IG10), slides were incubated with a secondary antibody followed by alkaline phosphatase-streptavidin and chromogen (DAB).



Lane 1: BSA (400 ng) Lane 2: 4-HNE-BSA (100 ng) Lane 3: 4-HNE-BSA (200 ng) Lane 4: 4-HNE-BSA (400 ng)

Western blot of 4-HNE-modified BSA using HNEJ-1 Monoclonal Antibody (Clone IG10) at a 1:500 dilution.

WARNING
THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

#### WARRANTY AND LIMITATION OF REMEDY

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website

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### Description

4-hydroxy Nonenal (4-HNE) is a lipid peroxidation product that reacts with lysine, cysteine, or histidine residues in proteins. It is found in all tissues and serum, and the formation of 4-HNE-modified proteins is associated with oxidative stress. 4-HNE can ligate to and inactivate cytosolic and mitochondrial proteins, leading to mitochondrial dysfunction, loss of cell proliferation and differentiation, increased intracellular calcium levels and production of pro-inflammatory cytokines, and apoptosis. 4-HNE levels are negatively regulated by aldehyde dehydrogenases (ALDHs), glutathione-S-transferases (GSTs), and aldo-keto reductases (AKRs). Increased levels of 4-HNE protein adducts are found at the cell membrane, in the mitochondria, and in the cytosol during ferroptosis in vitro.<sup>2</sup> 4-HNE reduces mitochondrial oxygen consumption and membrane potential, as well as increases intracellular levels of reactive oxygen species (ROS), in vitro.<sup>3</sup> 4-HNE protein adducts increase in an age-related manner in the kidney, spleen, ovary, and uterus, but not the liver, in rats.<sup>2</sup> 4-HNE protein adducts are increased and GST levels are decreased in the brain of older mice in a Tg2576 mouse model of Alzheimer's disease, and increased levels of 4-HNE-modified proteins, including manganese superoxide dismutase (Mn-SOD), α-enolase, and malate dehydrogenase, are found in the inferior parietal lobule in postmortem brain from patients with early Alzheimer's disease.<sup>4,5</sup> HNEJ-1 has high affinity for 4-HNE modifications at lysine, cysteine, or histidine residues, whereas HNEJ-2-5 have varying affinities for Ivsine and cysteine adducts.<sup>6</sup> Cayman's HNEJ-1 Monoclonal Antibody (Clone IG10) can be used for immunofluorescence (IF), immunohistochemistry (IHC), immunoprecipitation (IP), and Western blot (WB) applications.

#### References

- 1. Breitzig, M., Bhimineni, C., Lockey, R., et al. 4-Hydroxy-2-nonenal: A critical target in oxidative stress? *Am. J. Physiol. Cell Physiol.* **311(4)**, C537-C543 (2016).
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- 3. Galam, L., Failla, A., Soundararajan, R., et al. 4-hydroxynonenal regulates mitochondrial function in human small airway epithelial cells. *Oncotarget* **6(39)**, 41508-41521 (2015).
- Benedetti, E., D-Angelo, B., Cristiano, L., et al. Involvement of peroxisome proliferator-activated receptor β/δ (PPAR β/δ) in BDNF signaling during aging and in Alzheimer disease: Possible role of 4-hydroxynonenal (4-HNE). Cell Cycle 13(8), 1335-1344 (2014).
- 5. Reed, T.T., Pierce, W.M., Markesbery, W.R., *et al.* Proteomic identification of HNE-bound proteins in early Alzheimer disease: Insights into the role of lipid peroxidation in the progression of AD. *Brain Res.* **1274**, 66-76 (2009).
- 6. Ozeki, M., Miyagawa-Hayashino, A., Akatsuka, S., et al. Susceptibility of actin to modification by 4-hydroxy-2-nonenal. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **827(1)**, 119-126 (2005).