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# Orexin A

Cat.No. 389 004; Polyclonal Guinea pig antibody, 100 µl antiserum (lyophilized)

# **Data Sheet**

Reconstitution/ Storage	100 $\mu$ l antiserum, lyophilized. For <b>reconstitution</b> add 100 $\mu$ l H <sub>2</sub> O, then aliquot and store at -20°C until use. Antibodies should be stored at +4°C when still lyophilized. Do not freeze! For detailed information, see back of the data sheet.
Applications	WB: not tested yet IP: not tested yet ICC: not tested yet IHC: 1: 500 IHC-P: 1: 500
Immunogen	Synthetic peptide corresponding to AA 46 to 65 from mouse Orexin (UniProt Id: O55241)
Reactivity	Reacts with: human, rat, mouse (O55241). Other species not tested yet.
Specificity	Recognizes Orexin A with only minor cross-reactivity to the unprocessed precursor protein. Does not cross-react to Orexin B. K.O. validated

TO BE USED IN VITRO / FOR RESEARCH ONLY NOT TOXIC, NOT HAZARDOUS, NOT INFECTIOUS, NOT CONTAGIOUS

#### Background

Orexins, also referred to as **Hypocretins**, are produced by specialized neurons in the lateral hypothalamus, which project to many different regions of the brain. Orexin A (33 amino acids) and Orexin B (28 amino acids) are both produced from a single pre-pro-orexin polypeptide by a cascade of enzymatic reactions. They operate via binding to two closely related G protein-coupled receptors called Orexin receptor 1 and 2 (OxR 1 and OxR 2). OxR 1 is selective for Orexin A whereas OxR 2 binds both peptides with similar affinity.

The orexin system is conserved in mammals, and plays a central role in regulating feeding, sleep/wake cycles, arousal, energy expenditure, reward seeking, cognition, and stress responses. Defects in orexin signaling are involved in diet-induced obesity, diabetes, narcolepsy, panic anxiety disorder, depression, and addiction.

#### Selected References for 389 004

Orexin (hypocretin) participates in central autonomic regulation during fight-or-flight response.

Kuwaki T

Peptides (2021) 139: 170530.. IHC; tested species: mouse

Orexin neurons play contrasting roles in itch and pain neural processing via projecting to the periaqueductal gray. Kaneko T, Oura A, Imai Y, Kusumoto-Yoshida I, Kanekura T, Okuno H, Kuwaki T, Kashiwadani H Communications biology (2024) 71: 290. . IHC; tested species: mouse

Lesion of NPY Receptor-expressing Neurons in Perifornical Lateral Hypothalamus Attenuates Glucoprivic Feeding.

Choi PP, Wang Q, Brenner LA, Li AJ, Ritter RC, Appleyard SM Endocrinology (2024) 1655: . . IHC; tested species: rat

Esr1+ hypothalamic-habenula neurons shape aversive states.

Calvigioni D, Fuzik J, Le Merre P, Slashcheva M, Jung F, Ortiz C, Lentini A, Csillag V, Graziano M, Nikolakopoulou I, Weglage M, et al

Nature neuroscience (2023) 267: 1245-1255. . IHC; tested species: mouse

Hypothalamic orexinergic neurons modulate pain and itch in an opposite way: pain relief and itch exacerbation.

Kaneko T, Kuwaki T, Kashiwadani H

The journal of physiological sciences: JPS (2022) 721: 21. . IHC; tested species: mouse

#### **Selected General References**

The Orexin System and Hypertension.

Huber MJ et al. Cell. Mol. Neurobiol. (2017) PubMed:28349223

Basal Forebrain Cholinergic System and Orexin Neurons: Effects on Attention.

Villano I et al. Front Behav Neurosci (2017) PubMed:28197081

The hypocretin/orexin system in sleep disorders: preclinical insights and clinical progress.

Chow M et al. Nat Sci Sleep (2016) PubMed:27051324

Sleep disorders, obesity, and aging: the role of orexin.

Nixon JP et al. Ageing Res. Rev. (2015) PubMed:25462194

Roles of the orexin system in central motor control.

Hu B et al. Neurosci Biobehav Rev (2015) PubMed:25511388

Motivational activation: a unifying hypothesis of orexin/hypocretin function.

Mahler SV et al. Nat. Neurosci. (2014) PubMed:25254979

Orexin modulation of adipose tissue.

Perez-Leighton CE et al. Biochim. Biophys. Acta (2014) PubMed:23791983

Access the online factsheet including applicable protocols at https://sysy.com/product/389004 or scan the QR-code.



# FAQ - How should I store my antibody?

## **Shipping Conditions**

 All our antibodies and control proteins / peptides are shipped lyophilized (vacuum freezedried) and are stable in this form without loss of quality at ambient temperatures for several weeks.

### Storage of Sealed Vials after Delivery

- Unlabeled and biotin-labeled antibodies and control proteins should be stored at 4°C before reconstitution. They must not be stored in the freezer when still lyophilized!
   Temperatures below zero may cause loss of performance.
- Fluorescence-labeled antibodies should be reconstituted immediately upon receipt. Long term storage (several months) may lead to aggregation.
- **Control peptides** should be kept at -20°C before reconstitution.

# Long Term Storage after Reconstitution (General Considerations)

- The storage freezer must not be of the frost-free variety ("no-frost freezer"). This cycle
  between freezing and thawing (to reduce frost-build-up), which is exactly what should be
  avoided. For the same reason, antibody vials should be placed in an area of the freezer that
  has minimal temperature fluctuations, for instance towards the back rather than on a door
  shelf.
- Aliquot the antibody and store frozen (-20°C to -80°C). Avoid very small aliquots (below 20 µl)
  and use the smallest storage vial or tube possible. The smaller the aliquot, the more the stock
  concentration is affected by evaporation and adsorption of the antibody to the surface of the
  storage vial or tube. Adsorption of the antibody to the surface leads to a substantial loss of
  activity.
- The addition of glycerol to a final concentration of 50% lowers the freezing point of your stock and keeps your antibody at -20°C in liquid state. This efficiently avoids freeze and thaw cycles.

# **Product Specific Hints for Storage**

#### Control proteins / peptides

• Store at -20°C to -80°C.

#### **Monoclonal Antibodies**

- Ascites and hybridoma supernatant should be stored at -20°C up to -80°C. Prolonged storage at 4°C is not recommended! Unlike serum, ascites may contain proteases that will degrade the antibodies.
- **Purified IgG** should be stored at -20°C up to -80°C. Adding a carrier protein like BSA will increase long term stability. Many of our antibodies already contain carrier proteins. Please refer to the data-sheet for detailed information.

#### **Polyclonal Antibodies**

- Crude antisera: With anti-microbials added, they may be stored at 4°C. However, frozen storage (-20°C up to -80°C) is preferable.
- Affinity purified antibodies: Less robust than antisera. Storage at -20°C up to -80°C is
  recommended. Adding a carrier protein like BSA will increase long term stability. Most of our
  antibodies already contain carrier proteins. Please refer to the data-sheet for detailed
  information.

#### Fluorescence-labeled Antibodies

• Store as a liquid with 1:1 (v/v) glycerol at -20°C. Protect these antibodies from light exposure.

# Avoid repeated freeze-thaw cycles for all antibodies!

# FAQ - How should I reconstitute my antibody?

#### Reconstitution

- All our purified antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add
  the amount of deionized water given in the respective datasheet. If higher volumes are
  preferred, add water as mentioned above and then the desired amount of PBS and a
  stabilizing carrier protein (e.g. BSA) to a final concentration of 2%. Some of our antibodies
  already contain albumin. Take this into account when adding more carrier protein.
   For complete reconstitution, carefully remove the lid. After adding water, briefly vortex the
  solution. You can spin down the liquid by placing the vial into a 50 ml centrifugation tube filled
  with paper.
- If desired, add small amounts of azide or thimerosal to prevent microbial growth. This is especially recommended if you want to keep an aliquot a 4°C.
- After reconstitution of fluorescence-labeled antibodies, add 1:1 (v/v) glycerol to a final
  concentration of 50%. This lowers the freezing point of your stock and keeps your antibody in
  liquid state at -20°C.
- Glycerol may also be added to unlabeled primary antibodies. It is a suitable way to avoid freezethaw cycles.
- Please refer to our tips and hints for subsequent storage of reconstituted antibodies and control peptides and proteins.