

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Nesfatin-1/Nucleobindin-2 in direct ELISAs and Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human Nesfatin-1/Nucleobindin-2 Pro26-Leu106 Accession # P80303
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

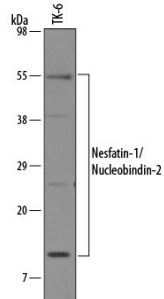
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below

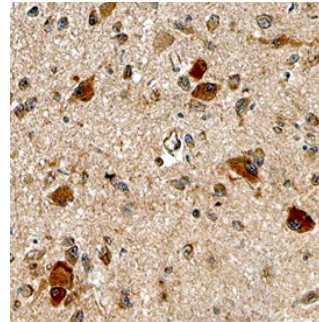
DATA

Western Blot



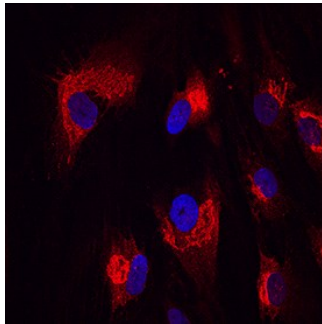
Detection of Human Nesfatin-1/Nucleobindin-2 by Western Blot. Western blot shows lysates of TK-6 human lymphoblast cell line. PVDF Membrane was probed with 1 µg/mL of Sheep Anti-Human Nesfatin-1/Nucleobindin-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5949) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). Specific bands were detected for Nesfatin-1/Nucleobindin-2 between 10 and 55 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Immunohistochemistry



Nesfatin-1/Nucleobindin-2 in Human Brain. Nesfatin-1/Nucleobindin-2 was detected in immersion fixed paraffin-embedded sections of human brain (hypothalamus) using Sheep Anti-Human Nesfatin-1/Nucleobindin-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5949) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to neuronal cell bodies. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

Immunocytochemistry



Nesfatin-1/Nucleobindin-2 in Human Mesenchymal Stem Cells. Nesfatin-1/Nucleobindin-2 was detected in immersion fixed human mesenchymal stem cells differentiated into adipocytes using Sheep Anti-Human Nesfatin-1/Nucleobindin-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5949) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to Golgi. View our protocol for [Fluorescent ICC Staining of Stem Cells on Coverslips](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Nesfatin-1 (NEFA Encoded Satiety and Fat-influencing protein 1) is a secreted, 10 kDa peptide derived from the translation product of the NUCB2 gene. Nesfatin-1 is associated with neurons involved in feeding (ARH and PVH) and fluid intake (SON and PVH), in β cells of the pancreas, and in adipocytes. Its presence peripherally has an anorexigenic effect. Mature human Nesfatin-1 is 82 amino acids (aa) in length. It is the secreted N-terminus of a larger, intracellular 420 aa precursor termed NEFA/Nucleobindin-2/NUCB2. NUCB2 contains a signal sequence (aa 1-24), Nesfatin-1 (aa 25-106), a DNA-binding site (aa 171-223), and two EF-hand regions (aa 241-276 and 293-328). Intracellular uncleaved NUCB2 is 45-50 kDa in size. There is a potential for multiple cleavages, generating Nesfatin-2 (aa 109-187) and Nesfatin-3 (aa 190-420). Human Nesfatin-1 shares 85% aa identity with mouse Nesfatin-1.