

Novocastra™ Lyophilized Rabbit Polyclonal Antibody Cholecystokinin (CCK-8)

Product Code: NCL-CCK-8p

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human cholecystokinin (CCK) and gastrin-containing cells in formalin-fixed, paraffin-embedded sections of human stomach (neuroendocrine cells). Specific staining is inhibited with sulfated CCK-8 and with human gastrin I. Cross-reactivity is observed with unsulfated CCK-8 and caerulein. Low cross-reactivity is observed with human gastrin I, CCK (30-33) and human big gastrin. No cross-reactivity is observed with pig vasoactive intestinal peptide (VIP).
Antigen Used for Immunizations	Synthetic sulfated cholecystokinin (26-33) amide (CCK-8) conjugated to keyhole limpet hemocyanin.
Preparation	Lyophilised rabbit serum diluted in PBS with 1% BSA containing 15 mM sodium azide. Reconstitute with 0.25 mL of sterile distilled water as indicated on vial label.
Effective on Frozen Tissue	Not evaluated.
Effective on Paraffin Wax Embedded Tissue	Yes
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:200–1:400. Trypsin digestion on paraffin sections is recommended 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Not recommended.
Positive Controls	Immunohistochemistry: Small intestine.
Staining Pattern	Cytoplasmic.
Storage and Stability	Store unopened lyophilized antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. The reconstituted antibody is stable for at least two months when stored at 4 °C. For long term storage, it is recommended that aliquots of the antibody are frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use.
General Overview	Cholecystokinin (CCK) was first isolated as a 33 amino acid intestinal peptide hormone which binds saturably and reversibly to distinct receptors in brain and pancreatic cell membranes. In both the brain and intestine, CCK exists in a number of molecular forms of which the C-terminal octapeptide (CCK-8) represents the most abundant molecular species. CCK, gastrin secretin and vasoactive intestinal polypeptide belong to the gastrointestinal hormone family. CCK functions to stimulate enzyme secretion from the pancreas, gall bladder contraction, intestinal motility, as well as inhibiting gastrin-induced acid secretion. CCK also serves as a neurotransmitter and modulates the action of other neurotransmitters, eg. dopamine, 5-HTGABA and excitatory amino acids. CCK is distributed in several regions of the brain including the cerebral cortex, hippocampus, amygdala nuclei and the hypothalamus. In the periphery, CCK is localized mainly in peripheral nerve fibers in the myenteric and submucosal ganglia as well as in endocrine cells of the gastrointestinal tract.
General References	Harro J, Vasar E and Bradwejn J. TIPS. 14: 244–249 (1993). Innis R B and Snyder S H. Proceedings of the National Academy of Sciences. 77 (11): 6917–6921 (1980). Vanderhaeghen J J, Lotstra F, De Mey J, et al.. Proceedings of the National Academy of Sciences. 77 (2): 1190–1194 (1980).



Instructions for Use

Trypsin Digestion for Immunohistochemical Demonstration on Paraffin Sections

1. Preheat the following to 37 °C using a water bath:
 - (i) 200 mL of TBS
 - (ii) 200 mL of distilled water.
2. Dissolve 0.2 g Trypsin 250 and 0.2 g Calcium chloride in the 200 mL of TBS.
3. Once the Trypsin solution is at 37 °C, pH to 7.8 with 1 M sodium hydroxide.
4. Place rehydrated paraffin sections in the distilled water to preheat the sections to 37 °C for a minimum of 5 minutes.
5. Incubate sections in Trypsin solution at 37 °C. The time required will depend on the antibody and tissue, however, 30 minutes is usually sufficient.
6. Rinse sections in running tap water.
7. Proceed with immunohistochemistry protocol.

Reagents Required but not Supplied

50 mM Tris-buffered saline

Trypsin 250: Difco order code 0152-13 (available from Becton Dickinson).

Calcium chloride

1 M Sodium Hydroxide

** Trypsin containing chymotrypsin should always be used. The enzyme activities can vary from a supplier and between batches. Such variations may affect the incubation time required.*