

**anti-CCAP**

anti-Crustacean Cardioactive Peptide
rabbit, polyclonal

Cat. No.	Amount
ABD-033	100 µl

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Molecular Weight: 956.13 g/mol

Purity: > 97 % (HPLC)

Form: liquid (Supplied in 10 mM sodium phosphate pH 7.4 and glycerol)

Description:

The crustacean cardioactive peptide (CCAP) is a potent cardioexcitatory substance, originally identified in the pericardial organs of the shore crab, *Carcinus maenas*. It also modulates the neuronal activity in other arthropods.

A CCAP-related new peptide family, the molluscan CCAP (M-CCAP) has been isolated and characterized from the snail *Helix pomatia* (Muneoka et al. 1994). Structural differences between the crustacean CCAP and the molluscan peptides are restricted only to the amidate-end of the molecules.

NOTICE

The anti-CCAP antiserum, generated against CCAP coupled to glutaraldehyde/polylysine (1:4), was tested for cross-reactivity using ELISA. No cross-reactivity was observed against 10 µg/ml of glutaraldehyde/polylysine conjugates of perisulfakinin, locustatachykinin II, FMRFamide, proctolin, adipokinetic hormone I, leucomyosuppressin, corazonin and the allatostatines, Dip-AST 2, Dip-AST 7, and Dip-AST 8.

Protocol for Crustacean Cardioactive Peptide (CCAP) detection by immunocytochemistry in invertebrate nervous system**Preparation**

Insects were cooled for 15 minutes and dissections were carried out in insect saline or in **solution A**. Ganglia or brain were exposed by opening and pinning out the dorsal cuticle, mounted dorsal-and in some cases ventral-side up on a wax coated glass disk.

Fixation

Cover up the insect brain or ganglia 30 min to 120 min with one of the Solutions B.

Vibratome sections

Immunocytochemistry was carried out on free-floating Vibratome sections by means of the indirect immunofluorescence immunocytochemistry. Brains or ganglia were wrapped in 5% agar and cut at 20-50 µm with a Vibratome

- in Solution C (for the fixation with solution B1) or
- in Solution D (for the fixation with solution B2) at 4°C.

Reduction Step

(optional and only for fixation with solution B1)

Vibratome sections are incubated during 10 min in the Solution C containing sodium borohydrite (0,1M) by stirring. Then, the tissue pieces are washed 5 times (15 min each) with Solution C by stirring. Sections are incubated during 12 hours at 4°C in Solution C + 30% sucrose.

Washing

Sections are washed 3 times (15 min each) in Solution C (for the fixation with Solution B1) and in Solution D (for the fixation with Solution B2) at room temperature.

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Application of antibody

The final dilution of the polyclonal anti-CCAP is 1:1000 in Solution C or D (depending on the fixation, see above) + 0.25 % Triton X100 + 1% goat serum + 3% milk powder (without fat) + 0,25 % BSA.

A dozen of sections can be incubated with 2ml of diluted antibody solution overnight or 48 h at 4°C by stirring. Then sections are washed 3 times 30 minutes with Solution D for both fixations by stirring.

Secondary Antibody

Sections are incubated with 1:600 dilution of Carbocyanin 3(Cy-3)-goat anti-rabbit complex (Jackson ImmunoResearch Laboratories, Inc.) in Solution D + 0.25 % Triton X100 + 3% milk powder (without fat) + 0,25 % BSA for 3 hours at 20°C by stirring.

SOLUTIONS TO BE PREPAREDSolution A

cacodylate 0.1 M, sodium metabisulfite 10g/l, pH 6.2*

Solution B1

(Boer-fixation) 15 ml aqueous saturated picric acid, 5 ml glutaraldehyde (25%), 0.1 ml glacial acetic acid

or

Solution B2

4% paraformaldehyde in Millonigs-phosphate buffer (pH 7.3-7.4, 1g NaCl, 2.9 g Na₂HPO₄*2H₂O, 0,524 g NaH₂PO₄*H₂O and 8 g paraformaldehyde were filled up to 200 ml with ddH₂O)

Solution C

Tris 0.05 M (Tris (hydroxymethyl) aminomethane), sodium metabisulfite 8.5 g/l, pH 7.5*

Solution D

Tris 0.05 M, NaCl 8,5 g/l, pH 7.5*

Adjust pH with NaOH or HCl if necessary

Tris solution can be replaced by a 0.01M phosphate solution.

Selected References:

Loesel *et al.* (2011) Neuroarchitecture of the arcuate body in the brain of the spider *Cupiennius salei* (Araneae, Chelicerata) revealed by allatostatin-, proctolin-, and CCAP-immunocytochemistry and its evolutionary implications. *Arthropod Struct Dev.* **40**:210.

Fort *et al.* (2007) Regulation of the Crab Heartbeat by Crustacean Cardioactive Peptide (CCAP):Central and Peripheral Actions.*J. Neurophysiol.* **97**:3407.

Vehovszky *et al.* (2005) Crustacean cardioactive peptide (CCAP)-related molluscan peptides (M-CCAPs) are potential extrinsic modulators of the buccal feeding network in the pond snail *Lymnaea stagnalis*.*Neurosci.Lett.* **373**:200.

Kirschnik *et al.* (2002) The influence of microgravity on the morphology of identified cerebral neurons in a cricket (*Acheta domesticus*). *J Gravit Physiol.* **9**:27.

Donini *et al.* (2001) Crustacean cardioactive peptide is a modulator of oviduct contractions in *Locusta migratoria*. *J Insect Physiol.* **47**:277.

Hernadi *et al.* (2000) The presence and specificity of crustacean cardioactive peptide (CCAP)-immunoreactivity in gastropod neurons. *Acta Biol Hung.* **51**:147.

Utting *et al.* (2000) Central complex in the brain of crayfish and its possible homology with that of insects. *J Comp Neurol.* **416**:245.