

anti-CCAP rabbit polyclonal antibody

Cat. No.	Amount
ABD-033	100 μ l

For *in vitro* use only
Quality guaranteed for 12 months.
Store at -20°C

AVOID FREEZE/THAW CYCLES.

Form

Liquid. Supplied in 10 mM Sodiumphosphate buffer pH 7.4, 50% glycerol.

Molecular weight CCAP

956.13

Purity

97.6% by HPLC

Description

The crustacean cardioactive peptide (CCAP) is a potent cardioexcitatory substance, originally identified in the pericardial organs of the shore crab, *Carcinus* means. 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 amidatedend of the molecules.

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

SOLUTIONS TO BE PREPARED

Solution 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 Millonig-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 phosphate solution 0.01 M.

IMMUNOCYTOCHEMISTRY on free-floating Vibratom sections

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

anti-CCAP rabbit polyclonal antibody

the dorsal cuticle, mounted dorsal-and in some cases ventral-side up on a wax coated glas disk.

Fixation

Cover up the insect brain or ganglia 30 min to 120 min with one of the **solutions**.

Vibratome section

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

· in **solution C** (for the fixation with **solution B1**)

or

· in **solution D** (for the fixation with **solution B2**), 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 mn each time) with the solution C, by stirring. The section are incubated during 12 hours, at 4°C, in the solution C + 30% of sucrose.

Washing

The sections are washed 3 times (15 mn each time) in **solution C** (for the fixation with **solution B1**) and in **solution D** (for the fixation with **solution B2**) at room temperature.

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 % of 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 the sections are washed 3 times, 30 minutes, with **solution D** for both fixations, by stirring.

Second 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 % of Triton X100 + 3% milk powder (without fat) + 0,25 % BSA for 3 hours at 20°C, by stirring.

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 $\mu\text{g}/\text{ml}$ of glutaraldehyde/polylysine conjugates of perisulfakinin, locustatachykinin II, FMR-Famide, proctolin, adipokinetic hormone I, leucomyosuppressin, corazonin and the allatostatines, Dip-AST 2, Dip-AST 7, and Dip-AST 8.

Selected References:

- Fort TJ, Garcia-Crescioni K, Agricola HJ, Brezina V, Miller MW. Regulation of the Crab Heartbeat by Crustacean Cardioactive Peptide (CCAP): Central and Peripheral Actions. *J Neurophysiol.* 2007 May;**97(5)**:3407-20.
- Vehovszky A, Agricola HJ, Elliott CJ, Ohtani M, Karpati L, Hernadi L. 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.* 2005 Jan 20;**373(3)**:200-5.
- Kirschnik U, Horn E, Agricola HJ. The influence of microgravity on the morphology of identified cerebral neurons in a cricket (*Acheta domestica*). *J Gravit Physiol.* 2002 Jul;**9(1)**:P27-8
- Donini A, Agricola H, Lange AB. Crustacean cardioactive peptide is a modulator of oviduct contractions in *Locusta migratoria*. *J Insect Physiol.* 2001 Mar;**47(3)**:277-285.
- Hernadi L, Agricola HJ. The presence and specificity of crustacean cardioactive peptide (CCAP)-immunoreactivity in gastropod neurons. *Acta Biol Hung.* 2000;**51(2-4)**:147-52.
- Utting M, Agricola H, Sandeman R, Sandeman D. Central complex in the brain of crayfish and its possible homology with that of insects. *J Comp Neurol.* 2000 Jan 10;**416(2)**:245-61.
- Mulloney B, Namba H, Agricola HJ, Hall WM. Modulation of force during locomotion: differential action of crustacean cardioactive peptide on power-stroke and return-stroke motor neurons. *J Neurosci.* 1997 Sep 15;**17(18)**:6872-83