

## anti-CCAP rabbit, polyclonal

Cat. No.	Amount
ABD-033	100 $\mu$ 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 and 50% glycerol.

### Molecular Weight CCAP

956.13g/mol

### 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 Millonigs-phosphate buffer (pH 7.3-7.4, 1g NaCl, 2.9 g Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, 0,524 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O and 8 g paraform-aldehyde were filled up to 200 ml with ddH<sub>2</sub>O)

#### 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.

### Purity

97% 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

#### 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 glas 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 Vibratom sections by means of the indirect immunofluorescence immunocytochemistry. Brains or ganglia were wrapped in 5% agar and cut at 20-50  $\mu$ m 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)

Vibratom 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.

### 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, FMR-Famide, proctolin, adipokinetic hormone I, leucomyosuppressin, corazonin and the allatostatines, Dip-AST 2, Dip-AST 7, and Dip-AST 8.

### Selected References:

- 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.
- Mulloney *et al.* (1997) Modulation of force during locomotion: differential action of crustacean cardioactive peptide on power-stroke and return-stroke motor neurons. *J. Neurosci.* **17**:6872.