

## CCAP antibody

Rabbit, Polyclonal

Cat. No.	Amount
<b>orb122519</b>	100 µl

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

### 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 allatostatins, Dip-AST 2, Dip-AST 7, and Dip-AST 8.

### 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 amidated end 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 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.



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### 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.

\* Adjust pH with NaOH or HCl if necessary

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

### 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.

### 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-rabbitcomplex 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 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\*



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