

anti-Proctolin rabbit, polyclonal

Cat. No.	Amount
ABD-032	100 μ l

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

Avoid freeze / thaw cycles

Form

Liquid.

Description

Polyclonal antibody against the neuropeptide proctolin.

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 Na₂HPO₄·2H₂O, 0.524g NaH₂PO₄·H₂O and 8 g paraformaldehyde were filled up to 200 ml with ddH₂O)

Solution C

0.05 M Tris, sodium metabisulfite 8.5 g/l pH 7.5*

Solution D

0.05 M Tris, NaCl 8.5 g/l pH 7.5*

Adjust pH with NaOH or HCl if necessary

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

Protocol - Proctolin 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 μ m with a Vibratome

·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 solution C containing sodium borohydrite (0,1M) by stirring. Then 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

The sections are washed 3 times (15 min 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 monoclonal anti-proctolin 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 antibody solution overnight or 48 h at 4°C, by stirring. Then the sections are washed 3 times 30 minutes each with solution D for both fixations by stirring.

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Secondary Antibody

Sections are incubated with 1:600 dilution of Carbocyanin 3(Cy-3)-goat anti-mouse 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-proctolin antiserum, generated against proctolin 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, crustacean cardiactive peptide, adipokinetic hormone I, leucomyosuppressin, corazonin and the allatostatines, Dip-AST 2, Dip-AST 7, and Dip-AST 8.

Selected References:

Eckert *et al.* (1981), Immunocytochemical identification of proctolin-like immunoreactivity in the terminal ganglion and hindgut of the cockroach *Periplaneta americana*. *Cell Tissue Res.* **217**:633.
Agricola *et al.* (1985), The distribution of a proctolin-like immunoreactive material in the terminal ganglion of the cockroach *Periplaneta americana*. *Cell Tissue Res.* **239**:203.
Clark *et al.* (2006), Proctolin-like immunoreactivity in the central and peripheral nervous system of the locust, *Locusta migratoria*. *Peptides* **27**:549.