

Conantokin-G and Fluorescent Conantokin-G Conjugates

C-6906 conantokin-G, tetramethylrhodamine conjugate

Quick Facts

Storage upon receipt:

- -20°C
- Avoid freeze-thaw cycles

Introduction

Conantokin-G, a peptide isolated from the venom of the cone snail *Conus geographus*, causes sleep aberrations in mice, including sleep-like symptoms in young mice and hyperactivity in older mice.^{1,2} This “sleeper peptide” is an antagonist of the *N*-methyl-D-aspartate (NMDA)-type of glutamate receptor.^{3,4} Synaptic activation of the NMDA receptor leads to an influx of Ca²⁺ that may mediate several neuronal processes, including long-term potentiation, neurite outgrowth, synaptogenesis and cell death.

Conantokin-G is a 17-amino acid peptide (2263 daltons) that contains five residues of the unusual amino acid λ -carboxy-glutamate (Gla): Gly-Glu-Gla-Gla-Leu-Gln-Gla-Asn-Gln-Gla-Leu-Ile-Arg-Gla-Lys-Ser-Asn-NH₂. Benke and colleagues prepared a tetramethylrhodamine derivative of conantokin-G and then used this modified peptide to determine the position and mobility of NMDA receptors in hippocampal neurons by confocal microscopy and digital imaging.⁴ For studies of the NMDA receptor, Molecular Probes offers unlabeled conantokin-G, as well as the red-orange fluorescent tetramethylrhodamine conjugates, which contain a single fluorophore per peptide.

Materials

Conantokin-G is supplied as a lyophilized powder in a unit size of 25 μ g. Tetramethylrhodamine conantokin-G are supplied as lyophilized powders in a unit size of 5 μ g. The lyophilized product should be stored desiccated at -20°C and protected from light. Allow product to warm to room temperature before opening the vial. Conantokin-G and tetramethylrhodamine conantokin-G

may be dissolved in any suitable buffer to yield an approximately 5 μ M (20–100X) stock solution; their molecular weights are 2263, ~2600 and ~2700 daltons, respectively. This stock solution should then be divided into single-use aliquots and stored frozen at -20°C. PROTECT FROM LIGHT. AVOID REPEATED FREEZING AND THAWING.

Application

It is a good practice to centrifuge the peptide conjugate solution briefly in a microcentrifuge before use; only the supernatant should then be added to the experiment. This step will eliminate any peptide aggregates that may have formed in solution, thereby reducing nonspecific background staining.

Labeling Tissue Samples and Cultured Cells

To label tissue and cells with fluorescent conantokin-G, add an aliquot of the 5 μ M stock solution to the sample of interest, incubate, wash and examine. The following labeling protocol is provided as an example to guide the researcher in the development of procedures that are optimized for a particular experimental system.

To label cultured cells, Benke and co-workers washed hippocampal cells three times with ES, incubated the cells with 100–250 nM tetramethylrhodamine conantokin-G for 1 hour at 4°C and washed again with ES. Cells were then fixed with cold (-20°C) methanol, air dried, rinsed with 10% goat serum in phosphate-buffered saline, pH 7.4 (PBS) and incubated with rabbit polyclonal antibodies that recognize a microtubule-associated protein.⁴

Fluorescence Microscopy

Cells labeled with tetramethylrhodamine conantokin-G may be viewed by fluorescence microscopy using standard tetramethylrhodamine optical filters; the excitation/emission maxima of this tetramethylrhodamine conjugate are ~554/575 nm. Further information on optical filter sets can be found in our *Handbook of Fluorescent Probes and Research Products*, at our Web site (www.probes.com) or by contacting our Technical Assistance Department.

References

1. Neurosci Lett 118, 241 (1990);
2. Biochemistry 26, 8508 (1987);
3. J Biol Chem 268, 17173 (1993);
4. Proc Natl Acad Sci USA 90, 7819 (1993).

Product List Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
C-6906	conantokin-G, tetramethylrhodamine conjugate	5 µg

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