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(54) **HIGH THROUGHPUT ASSAYS FOR THE
PROTEOLYTIC ACTIVITIES OF
CLOSTRIDIAL NEUROTOXINS**

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530/344; 530/350; 424/239.1; 435/4; 435/7.71;
435/7.72; 435/219

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See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,605,809 A 2/1997 Packard et al.
5,965,699 A 10/1999 Bostian et al.

FOREIGN PATENT DOCUMENTS

WO WO 95/33850 * 12/1995
WO WO0192312 12/2001

OTHER PUBLICATIONS

Soleilhac et al. (1996) A sensitive and rapid fluorescence-
based assay for determination of tetanus toxin peptidase
activity. *Analytical Biochemistry* 241, 120-127.

Anne, C. et al. (2001) High-throughput fluorogenic assay for
determination of botulinum type B neurotoxin protease
activity. *Analytical Biochemistry* 291, 253-261.

Roques, B. P. et al. (2000) Mechanism of action of clostridial
neurotoxins and rational inhibitor design. *Biology of the
Cell* 92, 445-557.

* cited by examiner

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(57) **ABSTRACT**

In this application is described substrates for high-through-
put assays of clostridial neurotoxin proteolytic activities.
Two types of substrates are described for use in assays for
the proteolytic activities of clostridial neurotoxins: (1) modi-
fied peptides or proteins that can serve as FRET substrates
and (2) modified peptides or proteins that can serve as
immobilized substrates. In both types a fluorescent mol-
ecule is present in the substrate, eliminating the require-
ment for the addition of a fluorogenic reagent. The assays
described can be readily adapted for use in automated or
robotic systems.

9 Claims, 3 Drawing Sheets