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(54) **METHODS, MICROFLUIDIC DEVICES, AND SYSTEMS FOR DETECTION OF AN ACTIVE ENZYMATIC AGENT**

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422/82

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See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,221,677	B1 *	4/2001	Wu et al.	436/518
6,488,872	B1	12/2002	Beebe	
2004/0009489	A1	1/2004	Golub	
2004/0048360	A1 *	3/2004	Wada et al.	435/287.2

OTHER PUBLICATIONS

A. S. Rathore; Cs. Horvath; "Axial Nonuniformities and Flow in Columns for Capillary Electrochromatography", Analytical Chemistry, 1998, 70, pp. 3069-3077.

L. J. Jin; B. C. Giordano; J. P. Landers; "Dynamic Labeling During Capillary or Microchip Electrophoresis for Laser-Induced Fluorescence Detection of Protein-SDS Complexes Without Pre- or Postcolumn Labeling", Analytical Chemistry, 2001, 73, pp. 4994-4999.

B. S. Broyles, S. C. Jacobsen; J. M. Ramsey; "Sample Filtration, Concentration, and Separation Integrated on Microfluidic Devices", Analytical Chemistry, 2003, 75, pp. 2761-2767.

T. Vilkner; D. Janasek; A. Manz; "Micro Total Analysis Systems. Recent Developments", Analytical Chemistry, 2004, 76, pp. 3373-3386.

A. V. Hatch; A. E. Herr; D. J. Throckmorton; J. S. Brennan; A. K. Singh; "Integrated Preconcentration SDS-PAGE of Proteins in Microchips Using Photopatterned Cross-Linked Polyacrylamide Gels", Analytical Chemistry, 2006, 78, pp. 4976-4984.

C. Yu; M. H. Davey; F. Svec; J. M. J. Frechet; "Monolithic Porous Polymer for On-Chip Solid-Phase Extraction and Preconcentration Prepared by Photoinitiated in Situ Polymerization within a Microfluidic Device", Analytical Chemistry, 2001, 73, pp. 5088-5096.

(Continued)

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(57) **ABSTRACT**

Embodiments of the present invention provide methods, microfluidic devices, and systems for the detection of an active target agent in a fluid sample. A substrate molecule is used that contains a sequence which may cleave in the presence of an active target agent. A SNAP25 sequence is described, for example, that may be cleaved in the presence of Botulinum Neurotoxin. The substrate molecule includes a reporter moiety. The substrate molecule is exposed to the sample, and resulting reaction products separated using electrophoretic separation. The elution time of the reporter moiety may be utilized to identify the presence or absence of the active target agent.

15 Claims, 10 Drawing Sheets

