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(54) **BUFFER SYSTEM FOR PROTEIN PURIFICATION**

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See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,634,678 A 1/1987 Salstrom
9,624,261 B2 * 4/2017 Goklen C07K 1/18
2003/0004094 A1 1/2003 Ghose et al.

2006/0118492 A1 6/2006 Shieh et al.
2008/0193981 A1 8/2008 Fahrner et al.
2008/0207879 A1 8/2008 Artur et al.
2009/0148435 A1 6/2009 Lebreton et al.
2009/0280131 A1 11/2009 DiPadova et al.
2009/0297620 A1 12/2009 Kanehira
2010/0234577 A1 9/2010 Mazzola et al.

FOREIGN PATENT DOCUMENTS

EP 0 363 126 A 4/1990
EP 0 617 049 B 9/1999
WO WO 2006/138553 A2 12/2006
WO WO 2010/063717 A1 6/2010
WO WO 2010/127069 A1 11/2010

OTHER PUBLICATIONS

Bjorck and Kronvall, J. Immunol., 133:969 (1984)).
Bjorck, J. Immunol., 140:1194 (1988).
Butler, J. N., Ionic Equilibrium: Solubility and pH Calculations. John Wiley and Sons (1998).
Forsgren and Sjoquist, J. Immunol., 97:822 1966.
Ghose, S.; McNeerney, T. M. Hubbard, B. pH transitions in ion-exchange systems: Role in the development of a cation exchange process for a recombinant protein. Biotechnol. Prog. 2002, 18, 530-537.
Mixed-mode Chromatography Selection Guide. <http://www.pall.com/main/laboratory/literature-library-details.page?id=47502> May 30, 2012.
Ngo, et al., Kosmotropes Enhance the Yield of Antibody Purified by Affinity Chromatography using Immobilized Bacterial Immunoglobulin Binding Proteins, Journal ofImmunoassay&Immunochemistry, 29:105-115,2008.
Pabst, T.M., Carta, G. pH transitions in cation exchange chromatographic columns containing weak acid groups. (2007) Journal of Chromatography A, 1142, pp. 19-31.
Soto Perez, J. and Frey, D. D. Behavior of the Inadvertent pH Transient Formed by a Salt Gradient in the Ion-Exchange Chromatography of Proteins, Biotechnol. Prog. 2005, 21, 902-910.
Yu, et al., Purification of hCG Monoclonal Antibodies by rProtein A Affinity Chromatography, Chinese Journal of Pharmaceuticals 2010, 41(8).
Zhou, J. X., et al. pH—conductivity hybrid gradient cation-exchange chromatography for process-scale monoclonal antibody purification. Journal of Chromatography A, 1175 (2007) 69-80.

* cited by examiner

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(57) **ABSTRACT**

A method for purifying a protein using a simplified, sodium chloride-free buffer system that consists of two components (acid and base pairs) for appropriate solution pH control; and a third component for ionic strength control, where the third component is the sodium salt conjugate base.

18 Claims, 6 Drawing Sheets