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**United States Patent** [19][11] **Patent Number:** **5,286,627**

Ueda et al.

[45] **Date of Patent:** **Feb. 15, 1994****[54] METHOD OF HIGH-SENSITIVE ANALYSIS OF BILE ACID AND REAGENT COMPOSITION FOR THE ANALYSIS****[75] Inventors:** Shigeru Ueda, Shizuoka; Masashi Tanno, Mishima; Hideo Misaki, Shizuoka, all of Japan**[73] Assignee:** Asahi Kasei Kogyo Kabushiki Kaisha, Osaka, Japan**[21] Appl. No.:** 510,716**[22] Filed:** Apr. 18, 1990**[30] Foreign Application Priority Data**

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**[51] Int. Cl.<sup>5</sup>** ..... C12Q 1/32; C12Q 1/26; C12N 9/02; C12N 9/08**[52] U.S. Cl.** ..... 435/26; 435/25; 435/189; 435/810**[58] Field of Search** ..... 435/25, 26, 14.4**[56] References Cited****U.S. PATENT DOCUMENTS**

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A method for a high-sensitivity quantitative analysis of bile acid in a bile acid-containing sample utilizes a reagent comprising:

(1) a steroid dehydrogenase which is capable of effecting a reversible reaction producing oxobile acid using bile acid, as a substrate, and a nicotinamide adenine dinucleotide phosphate compound (hereinafter referred to as an NADP compound) or a nicotinamide adenine dinucleotide compound (hereinafter referred to as an NAD compound) as coenzyme;

(2) a compound A<sub>1</sub> selected from the group consisting of NADP compounds and NAD compounds, in an amount surplus relative to the amount of bile acid;

(3) (i) compound B<sub>1</sub> selected from the group consisting of a reduced NAD compound and a reduced NADP compound, compound B<sub>1</sub> being a reduced NAD compound, when A<sub>1</sub> is an NADP compound, and a reduced NADP compound, when A<sub>1</sub> is an NAD compound, or

(ii) a compound B<sub>2</sub> which is an oxidized product of B<sub>1</sub>, or

(iii) a mixture of B<sub>1</sub> and B<sub>2</sub>, where the amount of B<sub>1</sub> plus B<sub>2</sub> is less than 1/100 of the molar amount of A<sub>1</sub>, and

(iv) a second dehydrogenase which does not react with bile acid and compound A<sub>1</sub>, but does effect a reaction converting compound B<sub>2</sub> into compound B<sub>1</sub>, and the substrate of the second dehydrogenase.

**18 Claims, 5 Drawing Sheets**