PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [7]: C07K 16/00, 16/30, C12N 15/13, 15/63 — A1

(11) International Publication Number: **WO 00/12556**

(43) International Publication Date: 9 March 2000 (09.03.00)

(21) International Application Number: PCT/GB99/02729

(22) International Filing Date: 20 August 1999 (20.08.99)

(30) Priority Data:
9818915.2 28 August 1998 (28.08.98) GB

(71) Applicant *(for all designated States except US)*: KS BIOMEDIX LTD. [GB/GB]; 42–46 High Street, Esher, Surrey KT10 9QY (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: HARRISON, Peter, John [GB/GB]; KS Biomedix Ltd., 42–46 High Street, Esher, Surrey KT10 9QY (GB).

(74) Agent: GILL JENNINGS & EVERY; Broadgate House, 7 Eldon Street, London EC2M 7LH (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HIGH–AFFINITY ANTIBODIES

(57) Abstract

High–affinity monoclonal antibodies, wherein the affinity is characterised by: (i) incubating first and second samples of the antibody in antigen–coated microtitre plate wells at a concentration chosen to be within the linear part of a standard curve at pH 7.2 for 1 hour at 37 °C; (ii) removing unbound antibody from both samples; (iii) incubating the first sample with PBS at pH 7.2 for 1 hour at 37 °C, and reducing the pH of the second sample to pH 3 or below and incubating for 1 hour at 37 °C; (iv) removing unbound antibody from both samples; (v) incubating both samples with anti–antibody alkaline phosphatase–conjugate for 1 hour at 37 °C; (vi) removing unbound conjugate from both samples; and (vii) adding PNPP substrate to the samples, measuring the absorbance of the samples at 405 nm, and determining the amount of antibody bound to antigen, wherein the amount bound in the second sample is > 50 % of that of the first sample.