

JUN 24 1997

K9604974

510(k) Summary

Submitter: DAKO Corporation
6392 Via Real
Carpinteria, CA 93013
(805)566-6655

Contact: Gretchen M. Murray, Ph.D., Regulatory Affairs Asst. Manager

Date Summary Prepared: June 19, 1997

Device Name: Mouse Anti-Human CD45/FITC, T29/33 +
Mouse Anti-Human CD14/RPE, TÜK4

Device Classification: Class II according to 21 CFR 864.5220, on the basis that monoclonal antibodies are accessories for automated differential cell counters.

Panel: This device classification is under the Hematology and Pathology devices panel, Division of Clinical Laboratory Devices.

Product Code: GKZ

Predicate Device(s): Becton Dickinson Simultest LeucoGATE

Device Description: Purified mouse anti-human CD45, Clone T29/33, conjugated with fluorescein isothiocyanate, isomer 1 (FITC) + purified mouse anti-human CD14, Clone TÜK4, conjugated with R-phycoerythrin, present in 0.05M Tris-HCl buffer, pH 7.2, 15 mM NaN₃, 0.1M NaCl, stabilized with 1% carrier protein

Subpopulations of lymphocytes may be stained with fluorochrome-conjugated antibody and evaluated in peripheral blood specimens when contaminating red blood cells (RBC's) are lysed prior to flow cytometric analysis. A subpopulation of WBC's are selected for assessment based upon cell morphology.

Intended Use: *For In Vitro* Diagnostic Use

Mouse Anti-Human CD45/FITC, T29/33 + Mouse Anti-Human CD14/RPE, TÜK4 (DAKO Anti-CD45/FITC and Anti-CD14/RPE) has been developed for use in flow cytometry to optimize the gating of lymphocytes when analyzing peripheral whole blood (erythrocyte lysed peripheral blood samples) or peripheral blood mononuclear cell preparations. It is one component of the suggested monoclonal antibody (MAb) combinations for routine immunophenotyping of lymphocytes in peripheral blood using flow cytometry.

Comparison of Technological Characteristics

Anti-CD45/FITC, T29/33, and Anti-CD14/RPE, TÜK4 is a dual antibody reagent in which each antibody has a related fluorescent conjugate. This reagent is used for detection and enumeration in peripheral blood. Clone T29/33 was clustered at the Third and Fourth International Leukocyte Workshops (In: McMichael, AJ, et al (Ed's). Leukocyte Typing III, Oxford, New York, Tokyo: Oxford University Press, 1987). Clone TÜK4 was clustered at the Fourth International Leukocyte Workshop (Knapp, W, et al (eds). Leukocyte Typing IV, Oxford, New York, Tokyo: Oxford University Press, 1989.)

Binding linearity was determined over serial dilutions of a cell line known to

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express the antigen diluted with a cell line that has no antigenic sites for each antibody. For Anti-CD45/FITC, the cell line with known antigenic reactivity is Raji cells, while the cell line without antigenic sites is U937 cells. For Anti-CD14/RPE, no cell line could be reliably grown for linearity testing. Linearity results are not available for this antibody. For Anti-CD45/FITC, five dilutions were tested, with linear equations calculated from the results. The equation for Anti-CD45/FITC, T29/33 was $y = 0.11\% + 0.997x$. $r = 0.999$.

Reproducibility of ten replicates from peripheral blood of one donor were run on two flow cytometers from different manufacturers. Different concentrations of antigen from whole blood was not possible to obtain.

	Mean % CD45 +	± 1 SD	%CV	n
Anti-CD45/FITC FACScan	99.8	0.1	0.1	10
	99.7	0.84	0.85	10
	100	0.04	0.04	10
Profile II	88.58	1.16	1.31	10
	95.61	1.18	1.24	10
	94.65	1.47	1.55	10
	Mean % CD14 +	± 1 SD	%CV	n
Anti-CD14/RPE FACScan	2.30	0.50	21.92	10
	2.63	0.41	15.46	10
	1.57	0.29	18.63	10
Profile II	3.02	0.26	8.66	10
	3.67	0.57	15.58	10
	6.41	0.42	6.60	10

Specificity of Anti-CD45/FITC, T29/33 in combination with Anti-CD14/RPE, TÜK4 has been verified by tests performed on five apparently healthy adult donors of various races at DAKO Corporation (3 Caucasians, 1 Asian, 1 Hispanic). Cell populations tested were RBC's, granulocytes, monocytes, lymphocytes and platelets. The results indicate antibody binding of Anti-CD45 T29/33 is specific for WBC's, not RBC's or platelets. Anti-CD14/RPE, TÜK4 is specific for monocytes, with some binding noted for granulocytes.

Anti-CD45/FITC with Anti-CD14/RPE Specificity

Averages (n = 5)	% Positive Red Blood Cells	% Positive Granulocyt es	% Positive Monocytes	% Positive Lymphocyt es	% Positive Platelets
CD45 ⁺ CD14 ⁺ Blood Cells	0.1 (0.0-0.2)	13.4 (1.9-35.4)	96.6 (93.7- 98.8)	0.2 (0.0-0.3)	0.1 (0.0-0.2)
CD45 ⁺ CD14 ⁻ Blood Cells	0.4 (0.1-1.1)	99.5 (98.0- 100.0)	99.3 (97.1- 100.0)	99.0 (96.9-99.9)	2.0 (0.4-4.5)
CD45 ⁻ CD14 ⁺ Blood Cells	0.2 (0.0-0.5)	13.5 (1.9-35.4)	96.6 (93.7- 98.8)	0.3 (0.0-0.5)	0.7 (0.4-1.2)

Correlation of the dual antibody reagent, Anti-CD45/FITC, T29/33, and Anti-CD14/RPE, TÜK4 to a predicate dual antibody reagent, Becton Dickinson Simultest LeucoGATE, was determined by testing duplicate samples with each reagent across 150 normal, apparently healthy individuals at three geographically separate laboratories and 27 samples obtained from ill patients. The regression analysis indicated that there was a 1:1 linear comparison of the DAKO CD45/CD14 to the LeucoGATE CD45/CD14 CD45/FITC. The linear equations that were generated are:

$$Y(\text{DAKO CD45/CD14 CD45+ cells}) = 18.68 + 0.81 X(\text{LeucoGATE CD45+ cells}), \\ r^2 = 0.8847$$

$$Y(\text{DAKO CD45/CD14 CD14+ cells}) = 0.44 + 0.91 X(\text{LeucoGATE CD14+ cells}), \\ r^2 = 0.6187$$

These equations indicate that the Anti-CD45/FITC, T29/33, and Anti-CD14/RPE, TÜK4 reagent and the Becton Dickinson Simultest LeucoGATE reagent are comparable on a 1:1 basis.

Conclusions:

Results of the above testing as well as the information provided by the Third, Fourth and Fifth Leukocyte Typing Workshops indicate that the DAKO Anti-CD45/FITC plus Anti-CD14/RPE reagent performs as well as Becton Dickinson's Simultest LeucoGATE in the detection and enumeration of CD45⁺ lymphocytes and the labeling of CD14 positive cells for the exclusion of these cells using flow cytometry for the detection and enumeration of lymphocytes. Safety of the DAKO Anti-CD45/FITC plus Anti-CD14/RPE reagent and its predicate device is high as all reagents are used for in vitro testing.



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Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Gretchen M. Murray, Ph.D.
Regulatory Affairs Asst. Manager
DAKO Corporation
6392 Via Real
Carpinteria, CA 93013

Re: K964974/S001
Trade Name: Monoclonal Mouse Anti-Human Leukocyte Common Antigen, CD45, FITC-conjugated, Clone T29/33 and Monoclonal Mouse Anti-Human Monocyte, CD14, RPE Conjugated, Clone T0K4
Regulatory Class: II
Product Code: GKZ
Dated: March 28, 1997
Received: April 04, 1997

Dear Dr. Murray:

We have reviewed your Section 510(k) notification of intent to market the device referenced above and we have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (Premarket Approval), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 895. A substantially equivalent determination assumes compliance with the Good Manufacturing Practice for Medical Devices: General (GMP) regulation (21 CFR Part 820) and that, through periodic GMP inspections, the Food and Drug Administration (FDA) will verify such assumptions. Failure to comply with the GMP regulation may result in regulatory action. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please note: this response to your premarket notification submission does not affect any obligation you might have under sections 531 through 542 of the Act for devices under the Electronic Product Radiation Control provisions, or other Federal Laws or Regulations.

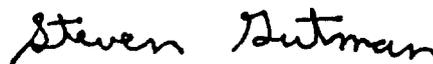
Under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88), this device may require a CLIA complexity categorization. To determine if it does, you should contact the Centers for Disease Control and Prevention (CDC) at (770)488-7655.

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This letter will allow you to begin marketing your device as described in your 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market:

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and additionally 809.10 for in vitro diagnostic devices), please contact the Office of Compliance at (301) 594-4588. Additionally, for questions on the promotion and advertising of your device, please contact the Office of Compliance at (301) 594-4639. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers Assistance at its toll free number (800) 638-2041 or at (301) 443-6597 or at its internet address "<http://www.fda.gov/cdrh/dsmamain.html>"

Sincerely yours,



Steven I. Gutman, M.D., M.B.A.
Director
Division of Clinical
Laboratory Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

10(k) Number (if known): K964974

Device Name: Monoclonal Mouse Anti-Human Leucocyte Common Antigen, CD45, FITC-conjugated, Clone T29/33 and Monoclonal Mouse Anti-Human Monocyte, CD14, RPE-Conjugated, Clone TUK4
Indications For Use:

Monoclonal Mouse Anti-Human Leukocyte Common Antigen, CD45, FITC-Conjugated, Clone T29/33 (Anti-CD45/FITC, T29/33) and Monoclonal Mouse Anti-Human Monocyte, CD14, RPE-Conjugated, Clone TUK4 (Anti-CD14/RPE, TUK4) have been developed for use in flow cytometry. This reagent may be used to optimize the gating of lymphocytes when analyzing peripheral whole blood or peripheral blood mononuclear cell preparations. This reagent is one component of the suggested monoclonal antibody (MAb) combination for routine immunophenotyping of lymphocytes in peripheral blood.

Lymphocyte gating is assigned by selection of the upper and lower channel numbers to define the lymphocyte population and is a quality control function for flow cytometry. Gating verification is performed by identification of 99±1% of the cells as CD45^{bright+} with CD14⁺ as 2% or less of the cell population.¹⁹

Note that this definition of "lymphocyte" does not give complete and unambiguous resolution from all other cell types. When optimizing light scatter gates for lymphocytes, operator and/or software algorithms are minimizing the number of lymphocytes excluded while still maintaining acceptable levels of contaminating cell types. Lymphocyte gate purity may be calculated by determining the percentage of non-lymphocyte events within the gate. Most non-lymphocytes are CD14 positive. Because each flow cytometer has different operating characteristics, each laboratory must determine its optimal operating procedure.

510(k) Number
Division of Clinical Laboratory Devices
(Division Sign-Off)
John E. Matus

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Concurrence of CDRH, Office of Device Evaluation (ODE)

Description Use
under 21 CFR 801.109)
D Use
under 21 CFR 801.119)

OR

Over-The-Counter Use _____
(Optional Format 1-2-96)