

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

**A. 510(k) Number:**

K060423

**B. Purpose for Submission:**

New Device

**C. Measurand:**

CD3, CD4, CD8 Lymphocytes

**D. Type of Test:**

Flow Cytometry, Quantitative and Semi-quantitative

**E. Applicant:**

DAKOCYTOMATION CALIFORNIA, INC.

**F. Proprietary and Established Names:**

CyAn™ DXD with Summit™ Software  
(Code CY204-30, CY205-30)

MultiMix™ Triple-Colour Reagent (Code TC660)  
Anti-Human CD8/FITC  
Anti-Human CD4/RPE  
Anti-Human CD3/APC

FluoroSpheres (Code K0110)

Anti-human CD3 FITC (Code F0818)  
Anti-human CD3 RPE (Code F0810)  
Anti-human CD3 APC (Code C7225)

**G. Regulatory Information:**

Product Code	Classification	Regulation Section	Panel
GKZ	Class II	864.5220	Hematology (81)

## H. Intended Use:

### 1. Intended use(s):

The CyAn™ DXD Flow Cytometer with Summit™ software and user manual is intended for use as an In-Vitro Diagnostic device for identification and enumeration of the relative fraction of lymphocyte subsets in human peripheral whole blood using flow cytometry, i.e., identifies the relative percentage of CD4 and CD8 T-cells as demonstrated by gating on CD3 positive cells. TC-660, the three color combination, CD3, CD4 and CD8 intended for use to identify the relative percentages of CD4 and CD8 positive T cells. Calibrators, FluoroSpheres (Dako K0110) are intended for in vitro use on the Dako CyAn™ DXD flow cytometer with Summit™ software to adjust detector voltages and monitor daily instrument performance. CD3 FITC (Dako F0818), CD3 RPE (R0810), and CD3 APC (C7225) single antibody- fluorochrome conjugates are intended to be used for setting fluorescence compensation parameters using automated compensation.

### 2. Indication(s) for use:

Clinical immunophenotyping using the CyAn™ DXD flow cytometer, a lyse wash sample preparation method, for identification and enumeration of CD3, CD4 and CD8 lymphocyte subsets using TC-660.

For In-Vitro Diagnostic Use

### 3. Special conditions for use statement(s):

Not applicable.

### 4. Special instrument requirements:

Not applicable.

## I. Device Description:

The Dako CyAn™ DXD device is a bench-top flow cytometer system relying on multiple (up to three) laser stimulation of fluorescence tagged lymphocytes. It is used with the Dako MultiMix, a triple color reagent; one each to CD3, CD4 and CD8, conjugated to fluorochromes APC(allophycocyanin), r-phycoerythrin, and fluorescein isothiocyanate, which are balanced to identify the dual positive T-cell populations (CD3+CD4+ and CD3+CD8+) in peripheral blood lymphocytes. The instrument requires daily set-up with Dako FluoroSpheres consisting of a set of 5 bead populations having different fluorescent intensities and one non-fluorescent bead population. The combination of fluorochromes enables excitation by light of any wavelength from 365-650 nm. The CyAn DXD utilizes anti-human CD3 conjugated with FITC, RPE and APC to perform autocompensation.

**J. Substantial Equivalence Information:**

Predicate	Item	Similarities	Differences
<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Intended Use</b>	The BD FACSCalibur IVD Flow Cytometer with CellQuest Software is intended for use as an In-Vitro Diagnostic device for identification and enumeration of the relative fraction of lymphocyte subsets in human cells in suspension using flow cytometry. I.e., identifies the relative percentages of CD4 and CD8 T-cells as demonstrated by gating on CD3 positive cells	The DakoCytomation CyAn IVD Flow Cytometer with Summit software is intended for use as an In-Vitro Diagnostic device for identification and enumeration of the relative fraction of lymphocyte subsets in human cells in suspension using flow cytometry I.e., identifies the relative percentages of CD4 and CD8 T-cells
<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Number of lasers</b>	2	3
<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Type of lasers</b>	488nm air cooled 635nm solid state diode	Solid state diode lasers 488nm OPSL 405nm diode 635nm semiconductor
<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Number and type of parameters</b>	<u>488 laser paths</u> FITC PE PE-TR/PerCP/PE-Cy5/PE-Cy7 <u>635 laser path</u> APC/APC-Cy7	<u>488nm laser path- 5 channels</u> FITC PE PE-Cy5 PE-Cy7 <u>635nm laser path</u> APC APC-Cy7 <u>405nm laser path</u> Pacific Blue
<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Calibrators</b>	BD CaliBRITE beads	FluoroSpheres (K0110) for instrument setup. CD3 FITC (DakoCytomation F0818), CD3 RPE (R0810), and CD3 APC (C7225) to set fluorescence compensation parameters
<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Reagents</b>	DC- FR875 (CD3 FITC/ CD4 PE) DC- FR881 (CD3 FITC/ CD8 PE)DC- FR700 (CD45 FITC/ CD14 RPE) DC-XO932 (IgG1 FITC/ IgG1 PE)	DC-TC660 (CD8 FITC/CD4 PE/CD3 APC) DC-CD3 Pacific Blue BD CD4 APC-Cy7 BD CD8 PE-Cy7 DC CD8 PE-Cy5

<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Software</b>	CellQuest/FACSCComp/Multiset	Summit v.4
<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Detectors</b>	1- Forward Scatter Photodiode 1-Side Scatter photomultiplier tube (PMT) 4- Fluorescence Detector PMTs	Forward Scatter Photodiode Side Scatter photomultiplier tube (PMT) 9 - Same 4 plus additional 5 Fluorescence Detector PMTs
<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Optics</b>	FACSCalibur Flow Cell Laser light delivered by mirrors, prisms, and lenses Emitted light delivered by mirrors	DakoCytomation Flow Cell Laser light delivered by mirrors, prisms, and lenses Emitted light delivered by mirrors
<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Electronics</b>	Analog	Analog/digital
<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Sample Introduction</b>	Manual sample loading	Same
<b>K974360 BD-FACS Calibur Becton Dickenson</b>	<b>Computer platform</b>	Macintosh	PC

**K. Standard/Guidance Document Referenced (if applicable):**

<b>STANDARDS</b>
<b>Title and Reference Number</b>
Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2)

GUIDANCE			
Document Title	Office	Division	Web Page
Draft Guidance for 510(k) Submission of Lymphocyte Immunophenotyping IVDs using Monoclonal Antibodies	OIVD	DIHD	<a href="http://www.fda.gov/cdrh/ode/475.pdf">http://www.fda.gov/cdrh/ode/475.pdf</a>

**L. Test Principle:**

TC660 consists of three monoclonal antibodies, each labeled with a unique fluorochrome. The CD8, CD4, and CD3 specific antibodies have been conjugated with FITC, RPE and APC, respectively. Lymphocyte subpopulations in peripheral blood samples may be stained with fluorochrome-conjugated antibody and evaluated after interfering red blood cells (RBCs) have been lysed.

An aliquot of a stained and lysed sample is applied to a flow cytometer and the cells flow in a single file past a laser beam. Stained cells will fluoresce depending on the nature of the fluorochrome label, FITC with an emission maximum at 520 nm, RPE at 575 nm and APC at 660 nm. This fluorescence as well as forward scatter (FSC) and side scatter (SSC) light is detected by the flow cytometer. The combination of fluorescence, FSC and SSC characteristics allows identification of T-cell subsets.

When excluding granulocytes and monocytes from the analysis gate on the basis of their FSC and SSC characteristics percent positive, lymphocytes can be determined. The T-cell counts are typically expressed as percentages of lymphocytes. Since each type of flow cytometer has different operating characteristics, each laboratory must determine its optimal operating procedure.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

*a. Precision/Reproducibility:*

Precision of the instrument was measured in two separate studies. In the first study, 5 whole blood specimens that had different ranges of CD3, CD4 and CD8 expression levels were used to measure precision for the FITC, RPE and APC parameters. Each specimen was separated in aliquots of 6, mixed with the test reagent and then acquired 10 times on the CyAn. The TC660 reagent (combined CD8 FITC/ CD4 RPE/ CD3 APC) was used as the detecting reagent. For the FITC parameter, standard deviations of 30 aliquots ranged from 0.18 to 0.88. For the RPE and APC parameters, standard deviations of 30 aliquots ranged from 0.17 to 1.02 and 0.25 to 0.93 respectively.

In the second study, cells obtained from JM and Raji cell lines were the test matrix. Cell line concentrations were 10%, 50% and 90% to test the middle and outer limits of

the instrument. Combination reagents consisting of CD3, CD4 or CD8 antibodies conjugated to different fluorochromes (APC-Cy7, RPE-Cy5, RPE-Cy7 and Pacific Blue) were used as the test reagents. Cells were mixed, separated in aliquots, and mixed with the antibody reagents. Each aliquot was acquired on the CyAn 10 times. Means, standard deviations and %CV's were determined. Means and standard deviations were determined as listed in table below:

Range of means and standard deviations for each parameter and cell line concentration:

% JM Cells	Reagent Pair 1		Reagent Pair 2	
	APC-Cy7 Means (standard deviations)	RPE-Cy7 Means (standard deviations)	RPE-Cy5 Means (standard deviations)	Pacific Blue Means (standard deviations)
90%	84.23 - 85.67% (0.38 - 0.94)	86.97 - 87.86% (0.94 - 1.18)	89.78 - 91.21% (0.46 - 0.90)	88.67 - 90.20% (0.60 - 0.75)
50%	46.00 - 47.93% (1.15 - 1.95)	46.75 - 49.93% (2.15 - 3.28)	50.76 - 52.54% (1.21 - 2.39)	48.46 - 50.95% (1.64 - 2.91)
10%	10.04 - 11.82% (0.39 - 0.67)	10.06 - 12.33% (0.63 - 1.13)	11.14 - 11.84% (0.37 - 0.69)	10.37 - 11.39% (0.46 - 0.99)

*b. Linearity/assay reportable range:*

For linearity, duplicate tubes of 5 different cell line concentrations ranging from 0 to 100% of positive cells were prepared. Each of 7 parameters was assessed for linear conformity to the theoretical 1:1 line. Linear equations for each parameter had a slope close to 1 and intercept close to 0, with an R<sup>2</sup> of at least 0.99.

Linearity results using cell line concentrations

	Intercept	Slope	R <sup>2</sup>
FITC	-1.79	1.04	0.9977
R-PE	-3.27	1.05	0.9942
APC	-2.74	1.04	0.9934
APC-Cy7	-1.38	1.05	0.9980
Pacific Blue	-1.01	1.04	0.9991
RPE-Cy5	-1.74	1.02	0.9993
RPE-Cy7	-2.95	1.02	0.997

Linear Range: 0-100%

Reportable Range: 0-100%0

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Reference material used for daily QC includes the three anti-CD3 reagents (CD3FITC, CD3RPE, CD3APC). Shelf-life studies for these reagents support three years for CD3FITC and two years for CD3RPE and CD3APC.

Based on manufacturer's supplied information on stability, the fluorospheres have a shelf life of two years. To verify this, a reference letter from Spherotech was included.

d. *Detection limit:*

Analytical limits are currently 0 and 100%.

e. *Analytical specificity:*

Not applicable

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Comparison testing was performed on a total of ninety-two (92) normal and abnormal specimens at three study sites. Regression analysis was performed comparing data obtained from the predicate and DakoCytomation FDA-cleared dual antibody reagents (CD45/CD14, CD3/CD4 and CD3/CD8) combination products to three different triple antibody-fluorochrome combination products run on the CyAn™ DXD instrument. Linear regression statistical analysis of the predicate device to test device comparison gave R2 values above 0.95 for all comparisons.

**Correlation of CD4 Positive Population**

<b>Linear regression equations and correlation statistic (R2) CD3+CD4+</b>			
Reagent Combination (CD3+CD4+)	CD8 FITC/CD4 RPE/ CD3 APC (TC660)	CD3 Pacific Blue/ CD4 APC-Cy7/ CD8 RPE-Cy5	CD3 Pacific Blue/ CD4 APC-Cy7/ CD8 RPE-Cy7
Linear equation	$y = 0.37 + 0.96b$	$y = -0.21 + 0.97b$	$y = 0.16 + 0.97b$
R2	0.9735	0.9744	0.9734
n	92	92	92

**Correlation of CD4 Negative Population**

<i>Linear regression equations and correlation statistic (R2) CD3+CD4-</i>			
Reagent Combination (CD3+CD4-)	CD8 FITC/CD4 RPE/ CD3 APC (TC660)	CD3 Pacific Blue/ CD4 APC-Cy7/ CD8 RPE-Cy5	CD3 Pacific Blue/ CD4 APC-Cy7/ CD8 RPE-Cy7
Linear equation	$y = 0.03+0.98b$	$y = 0.37+0.98b$	$y = 0.06+0.98b$
R2	0.9919	0.9914	0.9871
n	92	92	92

**Correlation of CD8 Positive Population**

<i>Linear regression equations and correlation statistic (R2) CD3+CD8+</i>			
Reagent Combination (CD3+CD8+)	CD8 FITC/CD4 RPE/ CD3 APC (TC660)	CD3 Pacific Blue/ CD4 APC-Cy7/ CD8 RPE-Cy5	CD3 Pacific Blue/ CD4 APC-Cy7/ CD8 RPE-Cy7
Linear equation	$y = -0.44+0.96b$	$y = 0.16+0.97b$	$y = -0.07+0.97b$
R2	0.9903	0.9916	0.9913
n	92	92	92

**Correlation of CD8 Negative Population**

<i>Linear regression equations and correlation statistic (R2) CD3+CD8-</i>			
Reagent Combination (CD3+CD8-)	CD8 FITC/CD4 RPE/ CD3 APC (TC660)	CD3 Pacific Blue/ CD4 APC-Cy7/ CD8 RPE-Cy5	CD3 Pacific Blue/ CD4 APC-Cy7/ CD8 RPE-Cy7
Linear equation	$y = 1.16+0.96b$	$y = 0.18+ 0.96b$	$y = 0.68+0.96b$
R2	0.9683	0.9735	0.9793
n	92	92	92

*b. Matrix comparison:*

Not applicable.

**3. Clinical studies:***a. Clinical Sensitivity:*

Not applicable.

*b. Clinical specificity:*

Specificity of MultiMix™ Triple-Colour Antibody Reagent Anti-CD8/FITC+Anti-CD4/RPE+Anti-CD3/APC were verified by tests performed on five apparently healthy adult donors of various races. Cell populations tested were lymphocytes, monocytes, granulocytes and debris (RBC, platelets). The results indicate antibody binding of MultiMix™ Triple-Colour Reagent Anti-CD8/FITC+Anti-CD4/RPE+Anti-CD3/APC is specific for T-lymphocytes.

**Specificity of triple-color reagent Anti-CD8/FITC+Anti-CD4/RPE+Anti-CD3/APC**

Range (n=5)	% Positive Lymphocytes	% Positive Monocytes	% Positive Granulocytes	% Positive Debris
CD3+CD4+	34.00-47.22	0.58-2.61	0.12-0.35	0.01-0.03
CD3+CD4-	20.08-33.46	.10-1.55	0.10-0.22	0.00-0.03
CD3+CD8+	19.08-37.68	0.35-1.56	0.15-0.26	0.00-0.04
CD3+CD8-	35.96-48.22	0.35-2.71	0.10-0.30	0.01-0.04

c. *Other clinical supportive data (when a. and b. are not applicable):*

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Blood samples were collected from 161 apparently healthy males and females at three geographically separate locations. The population included adults from a variety of races ranging in age from 20 to 69 years. Samples were stained with Anti-CD8/FITC, Anti-CD4/RPE and Anti-CD3/APC monoclonal antibodies. Normal CD3+CD4+, CD3+CD4-, CD3+CD8+, and CD3+CD8- T-cell values were measured by flow cytometry using the whole blood method, and are presented in the following table. Values below are expressed as a percentage of the total lymphocyte count due to unacceptable variation in absolute counts and are intended as representative values only. Each laboratory should establish its own expected values from the local population of normal donors.

	%CD3+/CD4+	%CD3+/CD8-	%CD3+/CD4-	%CD3+/CD8+
n	161	161	161	161
mean	44.58	25.85	23.11	47.33
St. Dev	7.67	7.21	6.24	7.40
%CV	17.21	27.88	26.99	15.63
5% Range	25.26	12.62	9.96	27.38
95% Range	62.28	42.30	37.73	63.42

**N. Instrument Name:**

CyAn™ DXD

**O. System Descriptions:**

1. Modes of Operation:

The modes of operation for the CyAn DXD are defined as Open Tube, Manual.

2. Software:

The CyAn Flow Cytometer requires use of the Summit software program to establish instrument settings, control acquisition, manipulate and save data, and provide other status and control regarding instrument behavior.

FDA has reviewed applicants Hazard Analysis and software development processes for this line of product types:

Yes   X   or No \_\_\_\_\_

3. Specimen Identification:

Patient data is manually entered.

4. Specimen Sampling and Handling:

Collect blood specimens by venipuncture into evacuated tubes containing EDTA, completely expending the vacuum in the tubes. Mix the blood well with the anticoagulant to prevent clotting. At least 100 µL of whole blood is required for each test. Ideally, blood samples should be processed immediately after collection. When this is not possible, each laboratory should validate that its collection and holding methods maintain specimen integrity comparable to freshly processed material. When using EDTA, blood samples should be held at room temperature (18-22 °C) and processed within 30 hours.

5. Calibration:

DakoCytomation K0110 FluoroSpheres are intended for in vitro use on the DakoCytomation CyAn DXD flow cytometer with Summit™ software to adjust detector voltages and monitor daily instrument performance. CD3 FITC (DakoCytomation F0818), CD3 PE (R0810), and CD3 APC (C7225) single antibody-fluorochrome conjugates are intended to be used for setting fluorescence compensation parameters.

6. Quality Control:

Daily Quality Control of the CyAn DXD is achieved through analysis of FluoroSpheres 6-peak beads (K0110) to ensure consistent resolution and sensitivity. Further, a normal specimen can be for an internal quality control.

**P. Other Supportive Instrument Performance Characteristics Data Not Covered In The Performance Characteristics Section above:**

Carryover testing was performed using 3 replicates of the 100% cell line concentrations followed by 3 replicates of 0% concentration of cell line cells. Parameters that were tested in the carryover study using cell lines included FITC, RPE, APC, Pe-Cy7, PE-Cy5, Pacific Blue, and APC-Cy7. Average carryover ranged from 0.45 to 1.18. The range found for the

0% cells after 100% cells were tested was 0.35 to 1.72. These levels remain within the known lower limits of detection of the instrument and these effects are considered minimal for the 7 parameters tested.

**Q. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**R. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

