CALTAG
LABORATORIES

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510(K) SUMMARY
SUMMARY OF SAFETY AND EFFECTIVENESS DATA

Caltag Cal-Lyse™ Lysing Solution For Use With Caltag Monoclonal Antibodies In Flow Cytometric Procedures

NAME AND LOCATION OF MANUFACTURER:

Caltag Laboratories, Inc.
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NAME OF CONTACT PERSON:

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DATE OF PREPARATION OF SUMMARY:

December 24, 1996

TRADE NAME OF THE DEVICE:

Caltag Cal-Lyse Lysing Solution For Use With Caltag Monoclonal Antibodies In Flow Cytometric Procedures

COMMON NAME:

Caltag Cal-Lyse Lysing Solution

CLASSIFICATION NAME:

Automated Differential Cell Coulter (21 CFR 864.5220)

LEGALLY MARKETED DEVICE (PREDICATE DEVICE) TO WHICH THE MANUFACTURER IS CLAIMING SUBSTANTIAL EQUIVALENCE:

The Caltag CD19 R-PE monoclonal antibody is substantially equivalent to the Coulter CD19 RD1 monoclonal antibody when red blood are lysed with the Cal-Lyse lysing solution in flow cytometric procedures.

The Caltag CD19 TRI-COLOR monoclonal antibody is substantially equivalent to the Coulter CD19 FITC monoclonal antibody when red blood are lysed with the Cal-Lyse lysing solution in flow cytometric procedures.

The Caltag CD19 R-PE monoclonal antibody is substantially equivalent to the Coulter CD19 FITC monoclonal antibody when red blood are lysed with the Cal-Lyse lysing solution in flow cytometric procedures.

The Caltag CD19 TRI-COLOR monoclonal antibody is substantially equivalent to the Coulter CD19 RD1 monoclonal antibody when red blood are lysed with the Cal-Lyse lysing solution in flow cytometric procedures.

The Caltag CD19 R-PE monoclonal antibody is substantially equivalent to the Caltag CD19 TRI-COLOR monoclonal antibody when red blood are lysed with the Cal-Lyse lysing solution in flow cytometric procedures.

DESCRIPTION OF THE DEVICE:

Caltag monoclonal antibodies bind to the surfaces of viable blood cells that express the corresponding antigens. To identify cells bearing these antigenic determinants, peripheral blood samples are incubated with fluorochrome-conjugated monoclonal antibodies. Cells are subsequently washed to remove unbound antibody. Prior to the removal of unbound antibody, Cal-Lyse lysing solution is added to lyse red blood cells. Cells may subsequently be washed, resulting in the elimination of red cell debris as well as unbound antibody.

Cal-Lyse lysing solution contains paraformaldehyde as fixative, and no additional fixation is required. Antibody-stained and fixed leukocytes are subsequently analyzed by flow cytometric methods.

INTENDED USE OF THE DEVICE:

Caltag Cal-Lyse is a lysis solution to enable the lysis of erythrocytes in samples of anticoagulated human peripheral blood. Cal-Lyse lysing solution is intended as an aid in the enumeration of leukocytes that have been stained with Caltag monoclonal antibodies for analysis by flow cytometric methods.

SUMMARY OF THE TECHNICAL CHARACTERISTICS OF THE MANUFACTURER'S DEVICE COMPARED TO THE PREDICATE DEVICE:

Comparisons of Caltag CD19 and Coulter CD19 Monoclonal Antibodies In Samples In Which Red Blood Cells Are Lysed With Caltag Cal-Lyse Lysing Solution

No.	Item	Caltag Antibodies	Coulter Antibodies	Comparison
1.	Intended Use	Flow Cytometry	Flow Cytometry Immunofluorescence	Substantially equivalent
2.	Specificity	CD19	CD19	Substantially equivalent
3.	Target cell	B lymphocyte	B lymphocyte	Substantially equivalent
4.	Chemical form	Monoclonal antibody	Monoclonal antibody	Substantially equivalent
5.	Fluorochromes	R-PE, TRI-COLOR	FITC, RD1	Substantially equivalent
6.	Available forms			
	FITC	liquid, PBS	lyophilized	Substantially
	PE	liquid, PBS	liquid, PBS	equivalent
	TRI-COLOR	liquid, PBS	not available	
7.	Sample prep. methods	whole blood	whole blood	Substantially equivalent
8.	Expected values from this study (n=155)			
	R-PE	5-21%	4-21% (RD1)	Substantially
	TRI-COLOR	4-24%	3-23% (FITC)	equivalent

NON CLINICAL TESTS SUPPORTING A DETERMINATION OF SUBSTANTIAL EQUIVALENCE:

EXPECTED VALUE DATA

Blood samples were collected from a total of 155 apparently healthy adult normal donors in an age range of 16 to 72, and a mean age of 41.

Samples were stained with Caltag monoclonal antibodies and red blood cells were lysed with Caltag Cal-Lyse lysing solution. Samples were collected and analyzed in each of three independent laboratories. An approximately equal number of males and females were collected and analyzed in each laboratory.

The normal donor population included members of differing ethnic origins, including adult Caucasian, Black, Oriental and Hispanic. Donors in geographically diverse areas of the United States, including the Western, Eastern and SouthCentral regions, participated in this study.

Summary of expected values for the CALTAG T and B cell monoclonal antibodies, CD3 FITC and CD19 R-PE, for all normal donors:

procedure	mean % positive	S.D.	Range ±2 S.D.	n
CD3 FITC	71.0	7.4	58-86	155
CD19 R-PE	13.0	4.2	5-21	155

Expected values for pediatrics and adolescents have not been established.
The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.

SPECIFICITY DATA

Blood samples were obtained from healthy normal donors of Caucasian, Black, Hispanic and Oriental ethnic origins. Samples of each donor were stained with Caltag monoclonal antibodies and red blood cells were lysed with Caltag Cal-Lyse lysing solution. Cells contained in the lymphocyte, monocyte and granulocyte regions were selected for analysis. Separate samples from the same donors were prepared for analysis of red blood cells and platelets and stained with each of the Caltag monoclonal antibodies.

The following specificity data were obtained with the Caltag T and B cell monoclonal antibodies, CD3 FITC and CD19 R-PE, following the lysis of red blood cells with Caltag Cal-Lyse lysing solution:

CD3 FITC

Ethnic Origin	Percent of Stained Cells Lymph.	Mono.	Gran.	Plt.	RBC
Caucasian	65.2	1.7	1.5	0.3	0.6
Caucasian	81.4	1.4	0.5	0.4	0.3
Hispanic	79.2	1.9	0.6	0.3	0.4
Oriental	81.2	1.3	0.9	0.2	0.4
Black	84.9	0.9	0.6	0.4	0.4
Mean	78.4	1.4	0.8	0.3	0.4
±1 S.D.	7.6	0.4	0.4	0.1	0.1

CD19 R-PE Ethnic Origin	Percent of Stained Cells				
	Lymph.	Mono.	Gran.	Plt.	RBC
Caucasian	18.0	0.6	0.9	0.5	0.5
Caucasian	13.3	1.1	0.8	0.3	0.7
Hispanic	12.2	0.7	0.8	0.4	1.0
Oriental	11.2	1.6	1.3	0.4	0.5
Black	14.6	0.0	0.5	0.6	0.9
Mean	13.9	0.8	0.9	0.4	0.7
±1 S.D.	2.6	0.6	0.3	0.1	0.2

Specific and/or nonspecific antibody Fc binding to monocytes in a patient sample can be excluded by proper gating on lymphocytes on the flow cytometer.

LEUKOCYTE RECOVERY DATA

This study was conducted on blood samples obtained from 5 normal donors consisting of representatives from Caucasian, Black, Hispanic and Oriental ethnic origins. An appropriate hematology analyzer was used enumerate leukocytes prior to, and immediately following the lysis of red blood cells with Caltag Cal-Lyse lysing solution and with the Ortho-mune™ Lysing Reagent. Leukocyte recovery from members of differing ethnic origins was comparable. Leukocyte recovery following lysis with Cal-Lyse and Ortho-mune lysing reagents was comparable.

It should be noted that washing of cells alone, in the absence of a lysis procedure may result in a modest loss of cells.

In the following table describing leukocyte recovery, leukocyte counts are expressed as cells per cu. mm.

Donor No.	Race	Leukocyte Count Prior to Lysis	Following Lysis	Percent Recovered
1	Caucasian	8300	7200	86.7
2	Caucasian	8100	7200	88.9
3	Black	5700	4800	84.2
4	Hispanic	6200	6000	96.8
5	Oriental	7400	7200	97.3
Mean		7140	6480	90.8
± 1 SD		1150	1073	6.0

RED BLOOD CELL LYSIS DATA

This study was conducted on blood samples obtained from 5 normal donors, to determine whether essentially all red cells were lysed by the lysing solution. Red cells were lysed in a sample of blood from each donor with Caltag Cal-Lyse lysis solution. An appropriate hematology analyzer was used to enumerate red blood cells prior to and immediately following lysis. The differing orders of magnitude of red cells counted prior to and following lysis should be noted in the following table.

Red Blood Cell Count

Donor No.	Prior to Lysis cells/cu.mm. x 10^6	Following Lysis cells/cu.mm x 10^5	Percent Lysed
1	5.1	3.0	94.1
2	5.1	5.0	90.2
3	4.3	3.0	93.0
4	3.7	3.0	91.8
5	4.9	5.0	89.7
Mean	4.6	3.8	91.8
±1 S.D.	0.6	1.1	1.9

CLINICAL TESTS SUPPORTING A DETERMINATION OF SUBSTANTIAL EQUIVALENCE:

CORRELATION DATA

The correlation for the Caltag Cal-Lyse lysing solution was based on the performance of the Caltag B cell monoclonal antibodies CD19 R-PE and CD19 TRI-COLOR. The percent, as well as the mean fluorescence, of B lymphocytes is substantially lower than is observed for T lymphocytes in normal peripheral blood. The resulting analysis of B lymphocytes by flow cytometric methods may be more susceptible to uncontrolled variations in the staining and lysis methods employed.

Samples obtained from normal and abnormal donors were stained with Caltag and comparable Coulter B cell monoclonal antibodies. Red blood cells were lysed with Caltag Cal-Lyse lysing solution in samples that had been stained with both Caltag and Coulter monoclonal antibodies.

A total of 155 normal donor samples were collected and analyzed in each of three independent laboratories, and analyzed on either the FACscan or Profile flow cytometers.

The normal donor population included members of differing ethnic origins, including adult Caucasian, Black, Oriental and Hispanic. Donors in geographically diverse areas of the United States, including the Western, Eastern and SouthCentral regions, participated in this study. Males and females were represented in approximately equal numbers.

A total of 20 abnormal donors were analyzed on both the FACscan and Profile flow cytometers at a single site. Correlations were obtained for the Caltag and Coulter B cell monoclonal antibodies for all normal and abnormal donors (n = 175). Red blood cells from all samples were lysed with Cal-Lyse lysing solution.

Comparison of the Caltag CD19 R-PE conjugated monoclonal antibody with the Coulter CD19 RD1 conjugated monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept	n
CD19 R-PE	16.4	97.7	0.92	1.30	175
CD19 RD1	16.2				

CD19 R-PE

Linear regression $y = 1.30 + 0.92x$

Comparison of the CALTAG CD19 R-PE conjugated monoclonal antibody with the Coulter CD19 FITC conjugated monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept	n
CD19 R-PE	16.4	96.3	0.93	0.69	175
CD19 FITC	16.7				

CD19 R-PE

Linear regression $y = 0.69 + 0.93x$

Comparison of the CALTAG CD19 TRI-COLOR conjugated monoclonal antibody with the Coulter CD19 RD1 conjugated monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept	n
CD19 TRI-COLOR	17.1	96.7	0.92	2.14	175
CD19 RD1	16.2				

CD19 TRI-COLOR

Linear regression $y = 2.14 + 0.92x$

Comparison of the CALTAG CD19 TRI-COLOR conjugated monoclonal antibody with the Coulter CD19 FITC conjugated monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept	n
CD19 TRI-COLOR	17.1	97.4	0.94	1.36	175
CD19 FITC	16.7				

CD19 TRI-COLOR

Linear regression $y = 1.36 + 0.94x$

Comparison of the CALTAG CD19 TRI-COLOR conjugated monoclonal antibody with the CALTAG CD19 R-PE conjugated monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept	n
CD19 TRI-COLOR	17.1	97.6	0.99	-0.56	175
CD19 R-PE	16.4				

CD19 TRI-COLOR

Linear regression $y = -0.56 + 0.99x$

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