MONOCLONAL ANTIBODIES TO HUMAN CELL SURFACE ANTIGENS

Mouse Anti CD8 Fluorescein-labeled (FITC)

CATALOG No. MHCD0801 100 tests 0.5 ml
CATALOG No. MHCD0801-4 400 tests 2.0 ml

Mouse Anti CD8 R-phycoerythrin-labeled (R-PE)

CATALOG No. MHCD0804 100 tests 0.5 ml
CATALOG No. MHCD0804-4 400 tests 2.0 ml

FOR IN VITRO DIAGNOSTIC USE
I. INTENDED USE

For In Vitro Diagnostic Use. CAUTION: Not for human or animal therapeutic use. Uses other than the labeled intended use may be a violation of local law.

II. SUMMARY AND EXPLANATION

The major cellular or cell-derived elements of human peripheral blood include lymphocytes, monocytes, granulocytes, red blood cells and platelets. The mature lymphocyte population contains functionally distinct cells types that are referred to as T (thymus-derived), B and Natural Killer (NK) cells. Two major subsets of T lymphocytes have distinctly different functional properties, and can be defined by correspondingly different cell surface glycoprotein antigens (1). The CD3+ mature T lymphocytes expressing the CD4 surface antigen are responsible for helper-inducer activity, while CD3+ cells that express the CD8 antigen have suppressor and cytotoxic activity (2).

The CD8 molecule is a 70 kDa glycoprotein dimer that is found on approximately 30% of peripheral blood T lymphocytes. This antigen is also expressed on most thymocytes that are located primarily in the cortical area of the thymus (3). The CD8 molecule contains one 32 kDa alpha chain and one 32 kDa beta chain. The chains may associate within the CD8 complex as a homodimer (alpha-alpha), or as a heterodimer (alpha-beta) (4).

The CD8 molecule is responsible for the recognition of class I major histocompatibility antigens (Class I MHC). The CD8+ cells include most cells expressing T-cytotoxic and T-suppressor activities (3,5) that have an important role in the regulation of the cellular immune response. A smaller percentage (5-15% of CD8+ cells have the morphology of large granular lymphocytes (LGL) and exhibit NK cell functions (6).

III. CLINICAL RELEVANCE

INVITROGEN’s CD8 monoclonal antibody recognizes the CD8 antigen (4). This antibody may be used, in combination with other indicators, for the diagnosis or prognosis of immunodeficiency diseases, including hypogammaglobulinemia, severe combined immunodeficiency (SCID) and the acquired immunodeficiency disease (AIDS) (7-11).

The CD8 monoclonal antibody may be useful in the identification of retroviral and other viral infections. An increase in the percentage of CD8+ cells has been observed in cytomegalovirus (CMV) and hepatitis B infections, as well in HIV disease (7-12).

IV. PRINCIPLES OF THE TEST

INVITROGEN’s FITC- and R-PE-labeled mouse monoclonal antibodies bind to the surfaces of viable cells that express the CD8 antigen. To identify cells bearing the CD8 antigenic determinant, peripheral blood leukocytes are incubated with the monoclonal antibodies and washed to remove unbound antibodies. Prior to removal of unbound antibodies, lysing solution is added to lyse red blood cells. An appropriate fixative solution is added to lysed and washed cells. Stained and fixed cells are subsequently analyzed by flow cytometric methods.

An appropriate isotypic control should be used in sample analysis to determine the amount of any background or nonspecific fluorescence. FITC- or R-PE-conjugated isotype controls should be of the same immunoglobulin isotype and of approximately the same antibody protein concentration as the CD8-conjugated monoclonal antibodies.
An appropriate cell gating control should be used in sample analysis. The CD45 pan lymphocyte and CD14 pan-monocyte reagents are intended to optimize the resolution of lymphocyte and monocyte populations (13).

V. REAGENTS

A. INVITROGEN MONOCLONAL ANTIBODY REAGENTS

Cluster Designation: CD8
WHO Classification: Leukocyte Workshop V (14)
Clone: 3B5
Isotype: IgG2a
Species: Mouse
Composition: IgG2a Heavy chain
Kappa Light chain
Source: Mouse ascites fluid
Method of Purification: Column chromatography
Fluorochrome: Fluorescein (FITC)
Excitation Wavelength 488 nm
Emission Wavelength 525 nm
R-Phycoerythrin (R-PE)*
Excitation Wavelength 488 nm
Emission Wavelength 575 nm
REAGENT CONTENTS

0.5 ml vial containing monoclonal antibody for 100 tests, in PBS buffer with BSA (and sucrose for RPE only), 0.1% sodium azide.

2.0 ml vial containing monoclonal antibody for 400 tests, in PBS buffer with BSA (and sucrose for RPE only), 0.1% sodium azide.

B. ADDITIONAL INVITROGEN MATERIALS REQUIRED

Isotype Control Reagent:

Mouse IgG2a FITC, 0.5 ml vial containing antibody for 50 tests, Catalog No. MG2a01

Mouse IgG2a R-PE*, 0.5 ml vial containing antibody for 50 tests, Catalog No. MG2a04

• U.S. Patent No. 4,520,110, Canadian Patent No. 1,179,942, European Patent No. 76,695

VI. STATEMENT OF WARNINGS

Reagents contain sodium azide. Sodium azide, under acid conditions, yields hydrazoic acid, an extremely toxic compound. Solutions containing sodium azide should be diluted with running water before being discarded. These conditions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Light exposure should be avoided. Use dim light during handling, incubation with cells and prior to analysis.

Do not pipet by mouth.

Samples should be handled as if capable of transmitting infection. Appropriate disposal methods should be used.

The sample preparation procedure employs a fixative (formaldehyde). Contact is to be avoided with skin or mucous membranes.

Do not use antibodies beyond the stated expiration dates of the products.

Deviations from the recommended procedure enclosed within this product insert may invalidate the results of testing.

FOR IN-VITRO DIAGNOSTIC USE

VII. APPROPRIATE STORAGE CONDITIONS

Store reagents at 2-8°C. Do not freeze. Reagents should be brought to room temperature (22±3°C) before use. Protect cells from light source during incubation with antibodies and prior to analysis.
VIII. EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration or substantial loss of reactivity is observed. The normal appearance of the FITC-conjugated monoclonal antibody is a clear, yellow liquid. The normal appearance of the R-PE-conjugated monoclonal antibody is a clear, pink-red liquid.

IX. SPECIMEN COLLECTION

Collect venous blood samples into blood collection tubes using an appropriate anticoagulant. For optimal results the sample should be processed within 6 hours of venipuncture. EDTA, ACD or heparin may be used if the blood sample is processed for analysis within 30 hours of venipuncture. ACD or heparin, but not EDTA may be used if the sample is not processed within 30 hours of venipuncture. Samples that cannot be processed within 48 hours should be discarded (15,16). If venous blood samples are collected into ACD for flow cytometric analysis, a separate venous blood sample should be collected into EDTA if a CBC is required. Unstained anticoagulated blood should be retained at 20-25°C prior to sample processing. Blood samples that are hemolyzed, clotted or appear to be lipemic, discolored or to contain interfering substances should be discarded.

Refer to "Standard Procedures for the Collection of Diagnostic Blood Specimens", published by the National Committee For Clinical Laboratory Standards (NCCLS) for additional information on the collection of blood specimens.

X. SAMPLE PREPARATION

1. Collect blood into an appropriate anticoagulant.
2. Determine leukocyte count and, if necessary, dilute blood in an isotonic solution to a concentration of approximately 5 x 10^6 leukocytes per ml. Determine cell viability using Trypan Blue or propidium iodide. If the cell viability is not at least 85%, the blood sample should not be used.
3. Pipette 100 µl of well mixed blood into 12 x 75 mm polypropylene centrifuge tubes marked Unknown and Control.
4. Add 5 µl of INVITROGEN’s CD8 FITC or CD8 R-PE conjugated monoclonal antibody to tubes marked Unknown. Add 5 µl of corresponding INVITROGEN IgG2a FITC- or IgG2a R-PE-conjugated isotypic control reagent to tubes marked Control. Mix gently.
5. Incubate all tubes for 15 minutes at room temperature (22±3°C) in the dark.
6. Add lysing solution to all tubes according to the manufacturer's directions.
7. Centrifuge all tubes at 400 x g for 3 minutes at room temperature.
8. Add fixing solution to all tubes according to the manufacturer's directions. Retain cells in fixing solution for not less than 30 minutes at room temperature (22±3°C) in the dark.
9. Wash the cells in all tubes twice with 4 ml of PBS. Centrifuge at 400 x g for 3 minutes after each wash procedure.
10. Resuspend the cells from the final wash in 1 ml of PBS and store tubes at 2-8°C in the dark until flow cytometric analysis is performed. It is recommended that analysis be performed within 18 hours of staining and fixation.
11. Analyze on a flow cytometer according to the manufacturer's instructions.
XI. MATERIALS REQUIRED BUT NOT SUPPLIED

Serofuge or equivalent centrifuge
12 x 75 mm polypropylene centrifuge tubes
Micropipette capable of dispensing 5 µl, 20 µl, 100 µl, and 500 µl volumes
Blood collection tubes with anticoagulant
Phosphate Buffered Saline (PBS)
Trypan Blue or propidium iodide, 0.25% (w/v) in PBS for the determination of cell viability (17)
Lysing solution
Fixing solution
Flow cytometer: Becton Dickinson FACScan™, Coulter® Profile® or equivalent

XII. INTERPRETATION OF RESULTS

FLOW CYTOMETRY

Analyze antibody-stained cells on an appropriate flow cytometer according to the manufacturer's instructions. The right angle or side light scatter (SSC) versus forward angle light scatter (FSC) is collected to reveal the lymphocyte cell cluster. A gate is drawn for the lymphocyte cluster (lymphocyte bitmap). The fluorescence attributable to the FITC- or R-PE-conjugated monoclonal antibody is collected, and the percentage of antibody-stained T lymphocytes is determined. An appropriate FITC- or R-PE-conjugated isotypic control of the same heavy chain immunoglobulin class and antibody concentration must be used to estimate and correct for nonspecific binding to lymphocytes. An analysis region is set to exclude background fluorescence and to include positively stained cells. The following histograms are representative of cells stained and gated on the lymphocyte region from a normal donor.

INVITROGEN IgG2a FITC ISOTYPE CONTROL
INVITROGEN CD8 FITC MONOCLONAL ANTIBODY

Fluorescence Intensity

Cell Number

INVITROGEN IgG2a R-PE ISOTYPE CONTROL

Fluorescence Intensity

Cell Number
INVITROGEN CD8 R-PE MONOCLONAL ANTIBODY

ABSOLUTE COUNTS

Use the following formula for the calculation of Absolute Counts:

\[ \text{Absolute Counts} = \text{Total White Cell Count} \times \% \text{ Positive Lymphocytes} \times \% \text{ Positively Stained Cells}/10^4. \]

Due to an unacceptable variance among the different laboratory methods for determining absolute counts, an assessment of the accuracy of the method is necessary (18).

XIII. QUALITY CONTROL PROCEDURES

Nonspecific fluorescence identified by the % cells stained to the right of the cursor on the histogram of the FITC-conjugated isotypic control is usually less than 2% in normal individuals. Nonspecific fluorescence identified by the R-PE-conjugated isotypic control is usually less than 4% in normal individuals. If the background level exceeds these values, test results may be in error. Increased nonspecific fluorescence may be seen in some disease states.

A blood sample from each normal and abnormal donor should be stained with the CD45 pan lymphocyte and CD14 pan monocyte monoclonal antibodies (13). When used in combination, these reagents assist in identifying the lymphocyte analysis region, and distinguish lymphocytes from monocytes, granulocytes and unlysed or nucleated red cells and cellular debris.
A blood sample from a healthy normal donor should be analyzed as a positive control on a daily basis or as frequently as needed to ensure proper laboratory working conditions. Each laboratory should establish its own normal ranges, since values obtained from normal samples may vary from laboratory to laboratory.

An appropriate isotype control should be used as a negative control with each patient sample to identify nonspecific Fc binding to lymphocytes. An analysis region should be set to exclude the nonspecific fluorescence identified by the isotypic control, and to include the brighter fluorescence of the lymphocyte population that is identified by the specific antibody.

Refer to the appropriate flow cytometer instrument manuals and other available references for recommended instrument calibration procedures (19, 20).

XIV. LIMITATIONS OF THE PROCEDURE

1. Incubation of antibody with cells for other than the recommended time and temperature may result in capping or loss of antigenic determinants from the cell surface.
2. The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.
3. Abnormal cells or cell lines may have a higher antigen density than normal cells. This could, in some cases, require the use of a larger quantity of monoclonal antibody than is indicated in the procedures for Sample Preparation.
4. Blood samples from abnormal donors may not always show abnormal values for the percentage of lymphocytes stained with a given monoclonal antibody. Results obtained by flow cytometric analysis should be considered in combination with results from other diagnostic procedures.
5. When using the whole blood method, red cells from some abnormal donors, as well as nucleated red cells found in normal and abnormal donors may be resistant to lysis by lysing solutions. Longer red cell lysing periods may be needed to avoid the inclusion of unlysed red cells in the lymphocyte gated region.
6. Blood samples should not be refrigerated or retained at ambient temperature for an extensive period (longer than 24-30 hours) prior to incubating with monoclonal antibodies.
7. Accurate results with flow cytometric procedures depend on correct alignment and calibration of the laser, as well as proper gate settings.
8. Due to an unacceptable variance among the different laboratory methods for determining absolute lymphocyte counts, an assessment of the accuracy of the method used is necessary (18).

XV. EXPECTED VALUES

Blood samples were collected from a total of 130 apparently healthy normal donors in an age range of 4 to 69. The population included members of differing ethnic origins, including adult Caucasians, Blacks, Asians and Hispanics, as well as pediatric and adolescent donors. Donors in geographically diverse areas of the United States, including the Eastern and Northcentral regions, participated in this study.

Blood samples collected from each donor were stained with INVITROGEN’s CD8 FITC or CD8 R-PE monoclonal antibodies.

Summary of expected values for INVITROGEN CD8 monoclonal antibodies for all normal donors:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Mean %</th>
<th>S.D.</th>
<th>Range</th>
<th>±2 S.D</th>
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<tbody>
<tr>
<td>Positive</td>
<td></td>
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</table>
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.

XVI. PERFORMANCE CHARACTERISTICS

SPECIFICITY

Blood samples were obtained from healthy normal donors of Caucasian, Black, Hispanic and Asian ethnic origins. Samples of each donor were stained with INVITROGEN’s CD8 FITC- or CD8 R-PE-conjugated monoclonal antibodies. Cells contained in the lymphocytes, monocytes and granulocytes 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 INVITROGEN monoclonal antibodies.

CD8 FITC

<table>
<thead>
<tr>
<th>Ethnic Origin</th>
<th>Percent of Stained Cells</th>
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<tr>
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<tr>
<td>Caucasian</td>
<td>28.7</td>
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<td>Caucasian</td>
<td>40.8</td>
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<tr>
<td>Hispanic</td>
<td>29.6</td>
</tr>
<tr>
<td>Asian</td>
<td>30.5</td>
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<tr>
<td>Black</td>
<td>25.5</td>
</tr>
<tr>
<td>Mean</td>
<td>31.0</td>
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<td>±1 S.D.</td>
<td>5.8</td>
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CD8 R-PE

<table>
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<tr>
<th>Ethnic Origin</th>
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<tbody>
<tr>
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<td>Caucasian</td>
<td>31.1</td>
</tr>
<tr>
<td>Caucasian</td>
<td>39.7</td>
</tr>
<tr>
<td>Hispanic</td>
<td>27.9</td>
</tr>
<tr>
<td>Asian</td>
<td>34.5</td>
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<tr>
<td>Black</td>
<td>30.4</td>
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<tr>
<td>Mean</td>
<td>32.7</td>
</tr>
<tr>
<td>±1 S.D.</td>
<td>4.6</td>
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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.

CORRELATION
Comparison of INVITROGEN’s CD8 FITC-conjugated monoclonal antibody with the GenClone 8 (CD8) FITC-conjugated monoclonal antibody:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Mean % Positive</th>
<th>S.D.</th>
<th>r value</th>
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<td>CD8 FITC</td>
<td>27.7</td>
<td>11.5</td>
<td>0.94</td>
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<tr>
<td>GenClone 8 FITC</td>
<td>28.2</td>
<td>10.8</td>
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</table>

**CD8 FITC**

Slope          +1.023  
y intercept    -1.104  
Linear regression  \( y = 1.023x -1.104 \)

Comparison of INVITROGEN’s CD8 R-PE-conjugated monoclonal antibody with the GenClone 8 (CD8) PE-conjugated monoclonal antibody:

<table>
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<th>Procedure</th>
<th>Mean % Positive</th>
<th>S.D.</th>
<th>r value</th>
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<td>CD8 R-PE</td>
<td>29.2</td>
<td>11.7</td>
<td>0.92</td>
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<tr>
<td>GenClone 8 PE</td>
<td>31.5</td>
<td>12.7</td>
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**CD8 R-PE**

Slope          +0.884  
y intercept    +1.344  
Linear regression  \( y = 0.884x +1.334 \)

REPRODUCIBILITY, INTRA-LAB

Intra-lab reproducibility for INVITROGEN’s CD8 FITC- and CD8 R-PE-conjugated monoclonal antibodies was determined by performing 15 replicated determinations for each antibody in each of three CD8⁺ ranges: high, medium and low. Thus, a total of 45 determinations were performed for each form of CD8. In this manner, reproducibility was demonstrated throughout the entire measuring range.

The 15 determinations for each range were performed by the staining, processing and analysis of 15 separate samples. Lymphocytes were selected for the analysis of percent cells stained in each of the three ranges.

To perform this study, anticoagulated blood was obtained from abnormal donors expressing a high percentage of CD8⁺ cells, or by depletion of CD4⁺ cells from normal donor blood using unconjugated CD4 monoclonal antibody immobilized to a solid support. Mid range and low range samples were obtained by mixing known CD8⁻ cells in appropriate ratios, while maintaining the same total cell concentration for the three ranges.

The study was performed in each of three independent laboratories, in the manner that each laboratory obtained, stained and analyzed separate blood samples. The following data are representative:
<table>
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<tr>
<th>Procedure</th>
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<th>S.D.</th>
<th>% CV</th>
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<td>27.4</td>
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<td>27.3</td>
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<td>3.7</td>
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**REPRODUCIBILITY, INTER-LAB**

Inter-lab reproducibility for INVITROGEN’s CD8 FITC and CD8 R-PE monoclonal antibodies was determined by performing 10 replicate determinations in each of three CD8⁺ ranges: high, medium and low. Thus, a total of 30 determinations were performed. In this manner, reproducibility was demonstrated throughout the entire measuring range.

The 10 determinations for each range were performed by the staining, processing and analysis of 10 separate samples. Lymphocytes were selected for the analysis of percent cells stained in each of the three ranges.

To perform this study, anticoagulated blood was obtained from a normal donor. The high range values were obtained by depletion of CD4⁺ cells using unconjugated CD4 monoclonal antibody immobilized to a solid support. Mid range and low range values were obtained by mixing known CD8⁻ cells in appropriate ratios, while maintaining the same total cell concentration for the three ranges.

The study was performed in each of three laboratories, in the manner that each laboratory stained and analyzed blood samples obtained from the same blood donor. The following data were obtained:
<table>
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<th>Procedure</th>
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<tr>
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<td>3.4</td>
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<td>22.7</td>
<td>0.7</td>
<td>3.2</td>
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SENSITIVITY STUDY

The measurement of sensitivity for INVITROGEN’s monoclonal antibodies was determined by staining a series of blood samples from a representative normal donor, with decreasing concentrations of each of the antibodies, so as to effect a titration curve relating the percent of lymphocytes stained to the reciprocal of antibody dilution.

The concentration ranges of antibodies were selected to include one or more antibody concentrations expected to saturate all epitopes on appropriate target cells (saturation point), and one or more lower antibody concentration expected to represent the least amount of antibody (detection threshold) that may be used to detect the same percentage of cells as was detected at the higher antibody concentrations. Thus a titration curve was obtained for each of the monoclonal antibodies to identify the saturation point and detection threshold. The study provides as a measurement of the potency of the antibodies for the intended target cells, and indicates the concentrations that are required to detect all target cells.

In practice, samples containing $10^6$ leukocytes/ml were stained with not less than 6 concentrations of each of the antibodies, in the range of 1.0 µg/test to 0.0005 µg/test.

Measurement sensitivity for INVITROGEN’s CD8 FITC monoclonal antibody:
Measurement sensitivity for INVITROGEN’s CD8 R-PE monoclonal antibody:

![Graph showing sensitivity of CD8 R-PE antibody](image)

**VII. BIBLIOGRAPHY**


### Explanation of symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Symbol</th>
<th>Description</th>
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<td>Catalogue Number</td>
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<td>IVD</td>
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<td></td>
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</tr>
<tr>
<td>[-]</td>
<td>Without, does not contain</td>
<td>[+ ]</td>
<td>With, contains</td>
</tr>
<tr>
<td>![Protect from light]</td>
<td>Consult accompanying documents</td>
<td>![Directs the user to consult instructions for use (IFU), accompanying the product.]</td>
<td></td>
</tr>
</tbody>
</table>