**F(ab')2TEST™ Anti-Human CD19/Light Chain Simultaneous Double Staining System**

Catalog No. AHM7010  50 Tests  3 Vial System

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**DESCRIPTION**

**Anti-Human CD 19 R-PE/Kappa FITC**

F(ab')2 monoclonal pan B cell marker anti-human CD 19 conjugated to R-Phycoerythrin* (R-PE) pre-mixed with fluorescein-conjugated goat polyclonal F(ab')2 antibodies to human kappa light chain.

**CONTROL**

Matched F(ab')2 monoclonal isotype (IgG1, Kappa) control conjugated to R-phycoerythrin* (R-PE) pre-mixed with fluorescein conjugated pre-immune polyclonal goat F(ab')2 antibody.

**Anti-Human CD 19 R-PE/Lambda FITC**

F(ab')2 monoclonal pan B cell marker anti-human CD 19 conjugated to R-Phycoerythrin* (R-PE) pre-mixed with fluorescein conjugated goat polyclonal F(ab')2 antibodies to human lambda light chain.

**NOTE:** All Double Staining System reagents are supplied as a purified immunoglobulin in a phosphate buffered saline, pH 7.2–7.4, containing stabilizing proteins and 0.1% sodium azide as a preservative.

<table>
<thead>
<tr>
<th><strong>ANTI-HUMAN CD 19</strong></th>
<th><strong>ANTI-LIGHT CHAIN</strong></th>
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<tbody>
<tr>
<td><strong>ORIGIN</strong></td>
<td>Clone SJ25-Cl, derived from hybridization of mouse SP2/0-Ag14 cells with spleen cells of Balb/c mice immunized with human cell line Nalm 1.</td>
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<td><strong>SPECIFICITY</strong></td>
<td>Anti-human CD 19 recognizes a 95 kD glycoprotein B cell restricted antigen designated CD 19.</td>
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<td><strong>REACTIVITY</strong></td>
<td>CD 19 is a pan B cell marker found throughout all stages of B cell development except plasma cells.</td>
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<td><strong>Ig CHAIN COMPOSITION</strong></td>
<td>F(ab')2 mouse IgG1 heavy chain, kappa light chain.</td>
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<td>Human kappa or lambda light chain immunized goats</td>
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<td>Kappa and lambda antibodies recognize human kappa or lambda surface Ig light chain, respectively.</td>
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<td>Surface immunoglobulin is expressed on mature resting and activated or proliferating B cells.</td>
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**RESEARCH APPLICATIONS**

Intended for use in the simultaneous detection of human pan B cell marker CD 19 and human light chain for direct determination of B cell surface Ig light chain phenotype by flow cytometry.

Determination of predominance of B cells expressing either kappa or lambda light chains as shown in the dot plots below.

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**WARNING**

This product contains sodium azide. To prevent the accumulation of explosive metal azides in plumbing, disposal must be in compliance with federal, state and local regulations pertaining to hazardous waste. For research use only. Not for use in diagnostic procedures.

*U.S. Patent # 4,520,110
HANDLING AND STORAGE

The reagents are provided such that 10 µL is sufficient to perform one test. One test is 100 µL of whole blood or 1 x 10⁶ cells. Vials contain reagent sufficient for 50 tests. Vials should be stored at 2 to 8°C and protected from prolonged exposure to light. **DO NOT FREEZE.** Do not dilute or aliquot reagents for storage. When stored as directed, the reagents are stable for the period shown on the label.

DIRECT IMMUNOFLUORESCENCE PROCEDURE FOR WHOLE BLOOD

PRECAUTIONS

- Handle all blood samples as if they are capable of transmitting infectious agents.
- Collect blood by venipuncture and anticoagulate with either ACD, EDTA or heparin. Proceed with sample staining within 6–8 hours of collection.

NOTE: These reagents may also be used to analyze density gradient-separated Peripheral Blood Mononuclear Cells (PBMCs). See Ficoll-Hypaque manufacturer's product insert for directions. Follow the "Staining Protocol" and then refer to the "Fixation and Storage" section below.

REQUIRED SUPPLEMENTARY MATERIALS

a) 12 x 75 mm disposable plastic or glass test tubes and test tube rack.
b) Phosphate buffered saline (PBS), pH 7.2–7.4, with 0.1% sodium azide.
c) Centrifuge, 2 to 8°C, 300 x g.
d) Micropipettes.
e) Phosphate buffered saline (PBS), pH 7.2–7.4, with 1% paraformaldehyde.
f) Erythrocyte lysing solution.
g) Flow cytometer.

PREPARATION OF WASHED WHOLE BLOOD CELLS

a) For each sample, pipet 100 µL anticoagulated whole blood into the bottom of three 12 x 75 mm plastic or glass test tubes.
b) Add 2–3 mL of PBS-azide to each tube and mix gently but thoroughly.
c) Pellet the cells by centrifugation for 5 minutes at 300 x g, aspirate supernate, taking care not to disturb the pellet.
d) Repeat steps B & C two more times (three washes).
e) When aspirating the last wash, leave approximately 200 µL of buffer over the cell pellet and gently resuspend the cells. (Gently shaking the test tube rack is sufficient.)

STAINING PROTOCOL

a) Add 10 µL of Anti CD 19 R-PE/Kappa FITC antibody to the cell suspension in one tube and vortex gently at low speed for 3 seconds.
b) Add 10 µL of Anti CD 19 R-PE/Lambda FITC antibody to the cell suspension in a second tube and vortex gently at low speed for 3 seconds.
c) A third aliquot of the cell suspension should be treated identically with control reagent.
d) Incubate cells protected from light for 30 minutes at either 2–8°C or room temperature.

Note: The dual staining reagents have been optimized on known positive chronic lymphocytic leukemia blood samples. Thus, 10 µL of reagent per test gives optimal results. To achieve optimal results with suspected normal blood samples, it is recommended that the laboratory dilute the F(ab′)₂TEST™ dual staining reagents 1:8 or 1:16 in PBS-azide.

ERYTHROCYTE LYSIS

a) Add the lysing reagent to the cells per manufacturer's directions, or your laboratory's normal procedure.
b) Follow the reagent manufacturer's recommended procedure or your usual procedure for time and temperature of incubation.
c) Following lysis, pellet the leukocytes by centrifugation for 5 minutes at 300 x g and carefully remove the supernate.
d) Wash the cells once in PBS-azide as described above.

FIXATION AND STORAGE

a) Gently loosen the cell pellet and resuspend in 250–500 µL PBS with 1% paraformaldehyde.
b) Maintain the stained cells at 2–8°C in the dark until analyzed, but no longer than 3 days.
c) Analyze on a flow cytometer, following the procedure supplied in the manufacturer's operating manual.
SELECTED REFERENCES


