

6602159 - 100 tests

6602173 - 25 tests

4235166-M



## POLYCLONAL (GOAT) ANTI-MOUSE ANTIBODY

### For In Vitro Diagnostic Use

#### INTENDED USE

COULTER CLONE® GAM-FITC antibody is intended for use as a second antibody detection reagent with fluorescence microscopy and flow cytometry methods to enumerate the percent of leukocytes which have reacted positively with mouse monoclonal primary antibodies in whole blood or Ficoll-Hypaque-separated cell suspensions.

#### SUMMARY AND EXPLANATION

A second antibody detection system labeled with fluorochrome (GAM-FITC) binds to an antigen-specific primary mouse monoclonal antibody which has been previously reacted with cell surface antigen. This results in increased sensitivity of the assay.

#### PRINCIPLES OF TEST

The use of this reagent depends on the ability of primary and secondary antibodies to bind to the surface of viable cells expressing discrete antigenic determinants.

Specific cell staining is accomplished by incubating peripheral blood leukocytes with a murine monoclonal primary antibody, washing the cells to remove unbound antibody, and then incubating them with COULTER CLONE® GAM-FITC reagent. Cells may then be enumerated by either fluorescence microscopy or flow cytometry.

#### REAGENTS

COULTER CLONE® GAM-FITC:

Coulter PN 6602159 - 100 tests, 6602173 - 25 tests

**SPECIFICITY:** COULTER CLONE® GAM-FITC antibody is a polyclonal goat antibody specific for the heavy and light chain activity against mouse IgG1, IgG2a, IgG2b, IgG3, and IgM.

**SOURCE:** Goat serum

**CONJUGATION:** FITC - Fluorescein Isothiocyanate

**MOLAR RATIO:** FITC/Protein - 2-4

#### FLUORESCENCE:

FITC (Green) Excites at 488 nm  
Emits at 518 nm

**PURIFICATION:** Ion exchange

**CYTOTOXICITY:** None by direct lysis

#### REAGENT CONTENTS

The final concentration of nonantibody reagents when reconstituted to 500 µL is 0.2% gelatin, 0.01 M potassium phosphate, 0.15 M NaCl, and 0.1% NaN<sub>3</sub>.

## STATEMENT OF WARNINGS

### For In Vitro Diagnostic Use

1. This reagent contains sodium azide. Sodium azide under acidic conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. This procedure requires the use of a fixative (2% formaldehyde). Inhalation or ingestion is harmful and may be fatal. If swallowed, induce vomiting. If skin or eye contact occurs, wash excessively with water.
3. Do not use product beyond the expiration date on label.
4. Patient specimens and all material coming in contact with them should be handled as if capable of transmitting infection, and disposed of with proper precautions.
5. Never pipet by mouth and avoid contact with skin and mucous membranes.
6. Do not expose reagents to strong light during storage or incubation.
7. Incubation times or temperatures other than those specified may give erroneous results.
8. Avoid microbial contamination of reagents or incorrect results might occur.
9. Harmful if swallowed.
10. After contact with skin, wash immediately with plenty of water.

## RECONSTITUTION

1. Reconstitute the lyophilized COULTER CLONE® monoclonal antibody, the isotopic control, and GAM-FITC by adding 500 µL of distilled water to each. This makes a stock solution.
2. Centrifuge the stock solution at 100,000 x g for 10 min for optimization of staining results.
3. Prepare working solutions\* of the above reagents as follows:

Vial Size	Volume of COULTER CLONE		Volume of PBS** Per Test
	GAM FITC	Stock Solution Per Test	
25 tests		20 µL	Add 180 µL
100 tests		5 µL	Add 195 µL

\*Diluted reagent must be used the same day as prepared.

\*\*PBS - Phosphate Buffered Saline

## STORAGE CONDITIONS

1. Unreconstituted lyophilized GAM-FITC product may be stored at 2 to 8°C for 5 years from date of manufacture. All reagents should be brought to 20-25°C prior to use.
2. Storage conditions of reconstituted GAM-FITC stock solution:  
Reconstituted and stored at 2-8°C - 6 months  
Reconstituted and stored at -70°C - 1 year
3. Avoid repeated freeze/thaw cycles. This will denature the antibody protein.
4. Do not store in a self-defrosting freezer.
5. Do not expose reagents to strong light during storage or incubation.
6. If all the reagent is not to be used within six (6) months, follow the Freezing Procedure below.

## FREEZING PROCEDURE FOR RECONSTITUTED PRODUCTS

### MATERIAL PROVIDED

COULTER CLONE® GAM-FITC:  
Coulter PN 6602159 - 100 tests,  
6602173 - 25 tests

## REAGENTS REQUIRED BUT NOT PROVIDED

Phosphate Buffered Saline (PBS): 0.145 M NaCl, 0.01 M potassium phosphate, pH 7.2 (Coulter PN 6602489)  
PBS containing 2% heat-inactivated fetal or newborn calf serum (2 mL calf serum diluted to 100 mL with PBS)

## PROCEDURE

Reconstitute the lyophilized COULTER CLONE® reagent by adding 500 µL of distilled water as described previously. Dilute the reconstituted COULTER CLONE® reagent with PBS containing 2% heat-inactivated calf serum prior to the freezing:

Vial Size	*Volume of Reconstituted Reagent/Test	**Volume of PBS With 2% Calf Serum/Test
25 tests	For each: 20 µL	90 µL
100 tests	For each: 5 µL	100 µL

\*These volumes may be frozen in multiple test volumes.

\*\*This yields one-half the working dilution of the monoclonal antibody.

Freeze the diluted reagent. Stable for one year when reconstituted, stabilized with a solution of fetal or newborn calf serum in PBS, and stored at -70°C. Do not freeze and thaw repeatedly. Store in aliquots.

Prior to use, allow the diluted reagent to reach room temperature. Dilute with the same volume as listed in the above table.

## EVIDENCE OF DETERIORATION

Any alteration of the physical appearance (pale green) of the reagent either lyophilized or reconstituted or any major variations in values on control subjects may indicate deterioration. If lyophilized reagents demonstrate evidence of rehydration prior to use, do not use.

## SPECIMEN COLLECTION AND PREPARATION

### SPECIMEN COLLECTION

Collect venous blood sample aseptically by venipuncture into VACUTAINER® tubes, or equivalent using an appropriate anticoagulant (EDTA is the anticoagulant of choice). For detailed information on the collection of whole blood by venipuncture and interfering conditions, refer to "Standard Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture (ASH-3)," published by the National Committee for Clinical Laboratory Standards. For each test, 100 µL of whole blood is required. Collect a sufficient amount of blood (1 to 2 mL required per tube) from each patient to run the test, control, and have autologous plasma for sample dilution. If necessary, a white blood cell count should be performed.

**CAUTION:** The stability of abnormal specimens is quite variable. For optimal results, start the assay within 6 hours of venipuncture. Unstained, anticoagulated blood should remain at 20 to 25°C until processing is begun. Do not refrigerate.

## SAMPLE PREPARATION

### Whole Blood

To prepare whole blood samples for analysis, follow the directions in the package insert:

COULTER Procedure for Indirect Immunofluorescence Cell Surface Staining with COULTER CLONE® Antibodies (Whole Blood Lyse), Coulter PN 4235444

### Ficoll-Separated Cell Suspensions

#### Materials Required But Not Provided

Flow cytometer or Fluorescence microscope.

See manufacturer's reference manual for details

Stopcock grease

Distilled or deionized water

Microscope slides (3" x 1" x 1.0 mm)

Coverslips (24 mm x 50 mm)

Venous blood sample

Phosphate Buffered Saline (PBS), 0.145 M NaCl, 0.01M potassium phosphate, pH 7.2 (Coulter PN 6602489)

Formaldehyde (10% solution) in PBS

Ficoll-Paque® (Pharmacia PN 17-0840-03)

PBS containing 2% fetal or newborn calf serum and 0.01% NaN<sub>3</sub> (wash media)

PBS containing 0.01% NaN<sub>3</sub> (resuspension media)

Propidium iodide 0.01 mg/mL, 0.05 mg/mL (Calbiochem PN 537059)

Acridine orange 0.005 mg/mL (Baker PN A366-3)

Low-speed refrigerated centrifuge with swinging bucket rotor

VACUTAINERS® tubes, or equivalent, with an appropriate anticoagulant

Siliconizing agent for glassware (Prosil®-28 Specialty Chemicals)

15-mL siliconized glass conical centrifuge tubes

12 x 75 mm siliconized glass or plastic standard test tubes

Transfer pipets

Ice bath

## PROCEDURE

1. Dilute 4 mL of blood 1:2 with 4°C PBS in an ice bath.
2. Layer 8 mL of diluted blood over 4 mL of Ficoll-Paque® in a 15-mL siliconized centrifuge tube.

**NOTE:** If a larger volume of cells is desired, any number of tubes of blood may be pooled into a large siliconized glass tube or flask and treated as above.

3. Centrifuge at 4°C at 400-450 x g for 30 min. Mononuclear cells should form a visible, clean interface between the plasma and the Ficoll-Paque®.
4. Aspirate the plasma and remove the mononuclear cell layer. Place cells in a clean 15-mL siliconized centrifuge tube. Fill the tube with 4°C PBS and gently mix the cells: spin at 400 x g for 8 min. Aspirate and discard the supernatant.
5. Resuspend cells in 4°C PBS, mix and spin at 400 x g for 4 min. Aspirate and discard the supernatant.
6. Resuspend cells in 4°C PBS, mix and spin at 400 x g for 3 min. Aspirate and discard the supernatant.
7. Resuspend cells with 5 mL of 4°C wash media or 4°C PBS, mix gently and determine cell concentration using a COULTER COUNTER® instrument (ZBI or S series) or hemocytometer.
8. Viability analysis is performed by one of the two following suggested methods (Fluorescence Microscopy or Flow Cytometry).

## Fluorescence Microscopy

Results obtained with fluorescence microscopes may vary due to the type of microscope used, the light source, the age of the bulb, filter assembly and filter thickness.

9. Place a suspension of approximately 25,000 cells (25 µL of a 1 x 10<sup>6</sup> cells/mL suspension) onto a microscope slide.
10. Add 10 µL of a 0.01 mg/mL solution of propidium iodide and mix gently by stirring with a pipet tip.
11. Allow to stand for 30 s. Add 10 µL of a 0.005 mg/mL solution of acridine orange. Mix gently and allow to stand for 3 s.
12. Place 24 x 50 mm coverslip on the slide, seal with stopcock grease, and examine by fluorescence microscopy.
13. Count 100 cells and report viability as the percent of viable cells which appear as bright green as opposed to nonviable cells which appear red. If viability is not 85%, the cell preparation should not be used.

## Flow Cytometry

Use an instrument that discriminates leukocytes and measures their fluorescence on a cell-by-cell basis.

9. Place 1 x 10<sup>6</sup> cells into a 12 x 75 mm siliconized test tube.
10. Wash one time with 4°C resuspension media and centrifuge at 400 x g for 30 min at 4°C.
11. Aspirate supernatant and add three drops of 0.05 mg/mL propidium iodide. Allow to stand for 1 min.
12. Wash two times in 4°C resuspension media as in step 10.
13. Aspirate supernatant and resuspend to 1 mL. Analyze on a flow cytometer system according to the instrument manual. If viability is not 85%, the cell preparation should not be used.

**CAUTION:** If the laser on the flow cytometer is misaligned or the gates are improperly set, results may be erroneous.

## PROCEDURES FOR INDIRECT IMMUNOFLUORESCENCE CELL SURFACE STAINING OF LEUKOCYTES WITH COULTER CLONE® MONOCLONAL ANTIBODY Whole Blood Reagent Preparation

Prepare the suspension of blood cells and stock solutions. (Refer to the Reconstitution and Specimen Collection and Preparation sections.) An appropriate isotypic control should be run with each patient sample, as well as a test tube containing cells and GAM-FITC only.

To stain whole blood samples for analysis, follow these steps:

1. Label the necessary siliconized tubes and add 200 µL of the appropriate monoclonal antibody working solution, isotype control or PBS to the corresponding tube.
2. Add 100 µL of the venous blood sample to each tube.
3. Vortex vigorously.
4. Incubate at room temperature for at least 10 min.
5. Wash with 4 mL of PBS, centrifuge at 400 x g for 3 min at 22-25°C. Aspirate supernate, vortex, and repeat.
6. Add 200 µL of GAM-FITC working solution to each tube.
7. Vortex vigorously.
8. Incubate at room temperature for at least 10 min. Incubation time may be increased to enhance fluorescence intensity.
9. Wash with 4 mL of PBS, centrifuge at 400 x g for 3 min at room temperature, aspirate supernatant carefully, and vortex vigorously. Repeat.
10. Add 1 mL of lytic reagent working solution to each tube; vortex vigorously and allow tubes to sit no less than 30 s, and no longer than 2 min before proceeding to step 11.
11. Add 250 µL of fixative (2% formaldehyde, see procedure in package insert, Coulter PN 4235444). Vortex vigorously.
12. Wash with 4 mL of PBS, centrifuge at 400 x g for 3 min at room temperature, aspirate supernatant carefully, and vortex vigorously. Repeat.
13. Add 1 mL of PBS and vortex.

## FICOLL-SEPARATED CELL SUSPENSIONS

### Reagents Required But Not Provided

Phosphate Buffered Saline (PBS): 0.145 M NaCl, 0.01 M potassium phosphate, pH 7.2 (Coulter PN 6602489)

PBS containing 2% heat-inactivated fetal or newborn calf serum (2 mL calf serum diluted to 100 mL with PBS)

PBS containing 0.01% NaN<sub>3</sub> (resuspension media)

10% formaldehyde in PBS

COULTER CLONE® Monoclonal Antibody

COULTER CLONE® Isotypic Control

Siliconizing agent for glassware (Prosil®-28 Specialty Chemicals)

## EQUIPMENT REQUIRED FOR TEST TUBE AND MICROTITER PLATE METHODS

Refrigerated centrifuge, capable of accurately achieving 400 x g

Ice bath

Fluorescence microscope and/or flow system

Ultracentrifuge (such as an airfuge)

Vortex mixer

### Test Tube Method Only

Centrifuge fittings - 12 x 75 mm test tube holders

12 x 75 mm siliconized glass or plastic test tubes

Transfer pipets

Coverslips (22 mm x 22 mm)

### Microtiter Plate Method Only

"V" bottom, vinyl, flexible, 96-well (8 x 12) microtiter plates

Plastic plate covers

Centrifuge carriers for microtiter plates

## REAGENT PREPARATION

Prepare the suspension of blood cells and stock solutions. (Refer to the Reconstitution and Specimen Collection and Preparation sections.) An appropriate isotypic control should be run with each patient sample.

## PROCEDURE

### Test Tube Method

1. Into 12 x 75 mm test tubes place 1 x 10<sup>6</sup> cells from Ficoll-Paque® preparation, centrifuge 4 min at 400 x g at 4°C, aspirate supernatant.
2. Add 200 µL of COULTER CLONE® monoclonal antibody working solution. Vortex gently. Incubate the reaction mixture at 4°C for 30 ± 5 min.
3. Wash each reaction mixture with 1 mL of 4°C wash media, centrifuge at 400 x g at 4°C for 4 min, aspirate supernatant carefully, and vortex gently. Repeat.
4. After the second wash, aspirate supernatant and add 200 µL of GAM-FITC working solution to the cell pellet. Disrupt pellet (vortex) and incubate at 4°C for 30 ± 5 min.
5. At the end of 30 min, wash three times with resuspension media, centrifuging each time for 4 min at 400 x g at 4°C.
6. After third wash, resuspend pellet to 1 mL with 4°C resuspension media for flow cytometry or fluorescence microscopy analysis.

### Microtiter Plate Method

1. Into each well place 1 x 10<sup>6</sup> cells.
2. Centrifuge microtiter plates at 4°C, at 400 to 450 x g for 5 min.
3. Remove the supernatant from each well by aspiration with a Pasteur pipet having a fire-polished and slightly bent tip. Insert the pipet tip into the well only as far as the lower ledge which permits efficient removal of all supernatant without disturbing the pellet.
4. Disrupt the cell pellets by carefully placing the lid on the tray and gently, vigorously pressing the microtiter plate "V" bottom onto the top of a vortex at an approximate setting of 8 or 9. All areas of the plate should be moved so that they come in contact with the vortex head. Vortex mixing should continue until all pellets are resuspended.
5. Add 200 µL of COULTER CLONE® monoclonal antibody and control working solutions into alternate wells and gently agitate. Incubate at 4°C for at least 30 min.
6. Centrifuge the plate at 4°C at 400 to 450 x g for 5 min.
7. Aspirate the supernatant and disrupt the pellets on the vortex mixer as in step 4.

8. Add 200 µL of wash media and centrifuge at 4°C at 400 to 450 x g for 5 min.
9. Aspirate the wash media and disrupt the pellets on the vortex mixer.
10. Add 200 µL of GAM-FITC working solution. Mix gently. Incubate the reaction mixture at 4°C for at least 30 min.
11. Centrifuge the plate at 4°C at 400 to 450 x g for 5 min. Wash the cell pellet twice as performed previously.
12. Resuspend the pellets in 200 µL of 4°C resuspension media and transfer to appropriate containers for flow cytometric analysis or fluorescence microscopy.

### Analysis of Cells by Manual Microscopy

1. Transfer 200 µL of each final cell suspension to test tube containing 20 µL of 10% formaldehyde in PBS. Place one drop of fixed cells on microscope slide. Cover with 22 mm x 22 mm coverslip avoiding entrapment of air bubbles. Seal with stopcock grease and examine by fluorescence microscopy.
2. Count all the cells in the field. Record the number of leukocytes by phase contrast differential. Examine the cells under fluorescent light (488 nm filter). Record the percentage of fluorescent leukocytes by switching between phase and fluorescent illumination.

### INTERPRETATION OF MICROSCOPY RESULTS

Counts are made of leukocytes (peripherally stained cells only). Three hundred leukocytes are counted and + (green) or - (not green) are enumerated.

$$\% \text{ positively-staining cells} = \frac{\text{No. of positive leukocytes}}{\text{Total leukocyte count (positive and negative)}} \times 100$$

This value expresses positive cells as % of leukocytes.

Analyze cells on a flow cytometer according to the instrument manual.

### QUALITY CONTROL PROCEDURE

A normal, apparently healthy donor should be run as a control to ensure proper working conditions. Tubes of patient cells stained with isotype control, antibody, and GAM-FITC (no first antibody) should be run to assess nonspecific staining.

An isotypic control is used to evaluate nonspecific binding for each patient sample. This staining is usually limited to 1-2% in normal individuals with higher values seen in some neoplastic diseases. If background levels of the control are not acceptable the test results of the patient sample should be considered invalid.

### LIMITATIONS

1. For optimal results, samples should be lysed within 6 h of collection. Retain samples in a vacutainer tube at room temperature prior to lysing and analyzing. Do not refrigerate. Stored or refrigerated blood samples may give aberrant results.
2. Immunodeficient patients may present special problems due to altered or very low numbers of certain lymphocyte populations.
3. Results are dependent on proper isolation of leukocytes. Prolonged contact of mononuclear cells with lymphocyte separation media may reduce cell viability. Cells should be removed and washed within five minutes after centrifugation. For whole blood samples, prolonged exposure of cells to the lytic reagent may cause white cell destruction.
4. Cells separated from whole blood by means of density gradients such as Ficoll-Paque® may not have the same relative concentrations of T and B cells as unseparated blood. This alteration is believed relatively insignificant for samples of blood from subjects with normal white blood counts. However, in

leukopenic patients or patients with low proportions of lymphocytes, the selective loss of specific subsets may affect the accuracy of the determination.

5. Incomplete gradient separation may occur in diseases marked by changes in leukocyte size or may be due to the separation technique. At times, a clear-cut interfacial layer of mononuclear cells may not appear following centrifugation, or the sample may have excessive erythrocytes, debris, immature myeloid cells, or granulocyte contamination. If this occurs, do not use the preparation. Redo the procedure.
6. Cryopreserved cells must have a viability of 85% to be used with these reagents or incorrect results may occur.
7. Abnormal states of health are not always represented by abnormal percentages of leukocytes. That is, an individual who may be in an abnormal state of health may exhibit the same leukocyte percentage as a healthy individual. Test results should be used in conjunction with information available from the clinical evaluation and other diagnostic procedures.
8. When using whole blood procedures, all red blood cells may not lyse under the following conditions: nucleated red cells, abnormal protein concentration or hemoglobinopathies. This may cause falsely decreased results due to unlysed red cells being counted as lymphocytes.

### PERFORMANCE CHARACTERISTICS SPECIFICITY

Each lot of GAM-FITC is carefully screened with each of our unconjugated monoclonal antibodies during the rigid quality control in-process testing protocols. The specimens used for this quality control testing are drawn from our employee population that includes Black, White, Oriental and Hispanic.

At the same time, any cross reactivity which could interfere with labeling to mouse antibodies is removed by solid phase (with human serum protein) adsorption technique against mouse IgG1, IgG2a, IgG2b, IgG3, and IgM, both heavy and light chain activity.

### SELECTED REFERENCE:

1. Bernard, A., et al.: 1984. Leucocyte Typing. Human Leucocyte Differentiation Antigens Detected by Monoclonal Antibodies. Springer-Verlag, New York, p 18.

### PRODUCT AVAILABILITY

COULTER CLONE® GAM-FITC Antibody  
Coulter PN 6602173 - 25 tests (0.5 mL)  
Coulter PN 6602159 - 100 tests (0.5 mL)

For additional information, call 1-800-526-7694.

### TRADEMARKS

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