

Whole Blood Lysing Reagents

REF 6602764 – 100 tests
REF 6603152 – 300 tests

PN 4235444-LD



For In Vitro Diagnostic Use

Rx Only

INTENDED USE

Whole Blood Lysing Reagents are used to prepare leukocytes from whole blood for immunofluorescence measurements on optical flow cytometers or fluorescence microscopes. The Whole Blood Lysing Reagents are reagents used to prepare leukocytes from whole blood for direct and/or indirect immunofluorescence cell surface staining with COULTER CLONE and CYTO-STAT/ COULTER CLONE Monoclonal Antibodies.

SUMMARY AND EXPLANATION

The ability to classify human white cells based upon their expression of cell surface antigens has provided investigators with valuable diagnostic and prognostic information.^{1,2} The Whole Blood Lysing Reagents were introduced for whole blood preparation for immunophenotyping.

PRINCIPLES OF TEST

The Whole Blood Lysing Reagents comprise a manual lysing system, which maintains leukocyte morphology and cell surface integrity. This method requires that the sample be washed.

The Whole Blood Lysing Reagents consist of IMMUNO-LYSE, an erythrocyte lytic agent, and Fixative, a cell membrane fixative. Both are available in the 100 test (PN 6602764) and 300 test (PN 6603152) kits. Stabilizer 1 for use with microscopic procedures is available only in the 100 test kit (PN 6602764).

REAGENTS

Whole Blood Lysing Reagents

REF 6602764 (100 tests)

For flow cytometry and fluorescence microscopy.

Whole Blood Lysing Reagents

REF 6603152 (300 tests)

For flow cytometry.

| Reagent | 100 Test Kit | 300 Test Kit |
|--------------|----------------|----------------|
| IMMUNO-LYSE | 1 x 6 mL Vial | 2 x 6 mL Vial |
| Fixative | 2 x 20 mL Vial | 1 x 75 mL Vial |
| Stabilizer 1 | 1 x 6 mL Vial | NA |

REAGENT CONTENTS

| | |
|---|-----------|
| IMMUNO-LYSE | |
| Sodium Azide | 1.0 mL/L |
| Fixative | |
| Formaldehyde | 92.5 g/L |
| Methanol | 37.5 mL/L |
| Stabilizer 1 | |
| Potassium Cyanide (buffered solution) | 3.3 g/L |

STATEMENT OF WARNINGS

| | |
|--|--|
| | DANGER |
| | Methanol <5% |
| | Formaldehyde 10 – 20% |
| | Potassium Cyanide <1% |
| | Ethoxylated Nonylphenol <3% |
| | Cetyldimethylethylammonium Bromide <5% |
| | Saponin 1 – 5% |
| | Harmful if swallowed. |
| | May be harmful in contact with skin. |
| | Causes severe skin burns and eye damage. |
| | Causes mild skin irritation. |
| | May cause an allergic skin reaction. |
| | Causes serious eye irritation. |
| | Suspected of causing genetic defects. |
| | May cause cancer. |
| | Causes damage to organs. |
| | Harmful to aquatic life with long lasting effects. |
| Avoid breathing vapours. | |
| Avoid release to the environment. | |
| Wear protective gloves, protective clothing and eye/face protection. | |
| IF ON SKIN (or hair): Rinse skin with water. | |
| IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. | |
| Immediately call a POISON CENTER or doctor/physician. | |
| If skin irritation occurs: Get medical advice/attention. | |
| If eye irritation persists: Get medical advice/attention. | |
| Take off contaminated clothing and wash it before use. | |
| Safety Data Sheet is available at techdocs.beckmancoulter.com . | |

- 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.
- Fixative contains 9.25% formaldehyde and 3.75% methanol. Avoid contact with skin and eyes as formaldehyde may cause irreversible effects to these tissues. Do not breathe vapors, harmful if swallowed. Wear appropriate safety equipment such as gloves and eye protection.
- Stabilizer 1 contains 0.33% Potassium Cyanide. Do not breathe vapors, harmful if swallowed. Do not mix or allow contact with water or acid. Wear appropriate safety equipment such as gloves and eye protection.
- IMMUNO-LYSE contains 0.1% sodium azide. Avoid contact with eyes. Reagents may irritate eyes or mucous membranes. If contact occurs, immediately flush with water and contact a physician. Do not mix or allow contact with water or acid.
- Specimens, samples and all materials coming into contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
- Never pipette by mouth and avoid contact with skin and mucous membranes.

- Do not use reagents beyond the expiration date printed on kit label.
- Incubation times or temperatures other than those specified may give erroneous results.
- Avoid microbial contamination of reagents or erroneous results may occur.
- Use Good Laboratory Practices (GLP) when handling these reagents.
- Harmful by inhalation, in contact with skin, and if swallowed.
- Irritating to eyes, respiratory system and skin.
- May cause sensitization by skin contact.
- Possible risk of irreversible effects.
- Limited evidence of a carcinogenic effect.
- Harmful if swallowed.
- After contact with skin, wash immediately with plenty of water.
- Keep away from sources of ignition - No smoking.
- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Wear suitable protective clothing, gloves and eye/face protection.
- Use only in well ventilated areas.
- Keep container tightly closed.

REAGENT PREPARATION

1. IMMUNO-LYSE

Working Solution: Make fresh working solution each day. Add 1 part IMMUNO-LYSE concentrate to 24 parts PBS to make a 1 to 25 dilution. One (1) mL of IMMUNO-LYSE working solution is required for each test. Prepare only the amount required for the assays to be performed.

2. Fixative

This reagent is used straight from the bottle. No dilution or reagent preparation is necessary.

3. Stabilizer 1

This reagent is used straight from the bottle. No dilution or reagent preparation is necessary.

STORAGE CONDITIONS AND STABILITY

Store at room temperature (22-28°C). Fixative may form a precipitate if stored at 2-8°C. If this occurs, place the bottle in a 37°C water bath until precipitate returns to solution. Unopened and opened reagent bottles are stable to the expiration date on the kit label.

EVIDENCE OF DETERIORATION

Any change in the physical appearance of the reagents, major variations in values for control subjects, or failure to lyse red blood cells in normal blood may indicate deterioration, and the reagents should not be used.

The normal appearance of IMMUNO-LYSE reagent is a clear, colorless to amber colored liquid.

The normal appearance of Fixative reagent is a clear, colorless liquid.

The normal appearance of Stabilizer 1 reagent is a clear, pink colored liquid.

SPECIMEN COLLECTION AND PREPARATION

CAUTION: The stability of blood samples is quite variable. For optimal lysis results, start the assay within 6 hours of venipuncture. Unstained, anticoagulated blood should remain at 20-25°C until processing begins. Do not refrigerate.

Collect a venous blood sample aseptically by venipuncture into a blood collection tube using an appropriate anticoagulant (EDTA is recommended).³ For each test, 100 µL of whole blood is required. Collect enough blood from each patient to run tests and controls. A white blood

cell count and cell viability should be performed for each venous blood specimen using an established laboratory procedure. Recommended cell viability is $\geq 90\%$, but this may be difficult to obtain with some abnormal specimens.

PROCEDURE FOR LEUKOCYTE PREPARATION USING WHOLE BLOOD LYING REAGENTS

MATERIAL SUPPLIED

Whole Blood Lysing Reagents Kit
[REF] 6602764 – 100 test

OR

Whole Blood Lysing Reagents
[REF] 6603152 – 300 test

MATERIALS REQUIRED BUT NOT SUPPLIED

Centrifuge fitted with 12 x 75 test tube holders

12 x 75 mm test tubes

Blood collection tubes with anticoagulant (EDTA is recommended)

Transfer pipettes

Vortex mixer

Micropipettors

Flow Cytometer

Phosphate Buffered Saline (PBS), pH 7.2, PN 6603369

PROCEDURE

Note: White blood cell counts outside the limits of 3,000-10,000 cells/ μL may require special treatment to adjust cell concentrations to the appropriate level. Autologous plasma is recommended for dilutions. Refer to the monoclonal antibody reagent package insert for recommended cellular concentrations.

PROCEDURE - INDIRECT STAINING

1. For each sample, label 12 x 75 mm test tubes for monoclonal antibody tests and appropriate isotypic controls. Add 200 μL of the appropriate amount of monoclonal antibody working solution to each tube. Refer to specific package inserts for complete reagent information including cell count adjustment.
2. Pipette 100 μL of anticoagulated whole blood into bottom of the properly labeled test tube. Be sure that the inside surface and top of the tube is free of blood. Vortex vigorously.
3. Incubate at room temperature for 10 minutes. (Incubations may be performed in an ice bath for enhanced fluorescence intensity.)
4. Wash two times with 4 mL of PBS, centrifuge at 400 x g for 3 minutes at room temperature, aspirate supernatant carefully. Vortex vigorously.
5. Add 200 μL of appropriate secondary antibody working solution to each tube. Vortex vigorously.
6. Incubate at room temperature for 5 minutes. (Incubation time may be increased to enhance fluorescence intensity.)
7. Wash two times with 4 mL of PBS, centrifuge at 400 x g for 3 minutes at room temperature, aspirate supernatant carefully, and vortex vigorously.
8. Add 1 mL of IMMUNO-LYSE working solution to each tube. Vortex vigorously.
9. Allow tubes to sit no less than 30 seconds, and no longer than 2 minutes before proceeding to the next step.
10. Add 250 μL of Fixative. Vortex vigorously.
11. Wash two times with 3 mL of PBS, centrifuge at 400 x g for 3 minutes at room temperature, aspirate supernatant carefully. Vortex vigorously.
12. Add 1 mL of PBS. Vortex vigorously.
13. Proceed according to the **Procedure-Fluorescence Microscopy** or **Flow Cytometric Results** heading.

PROCEDURE - FLUORESCENCE MICROSCOPY (PURIFIED AND FITC COULTER CLONE REAGENTS ONLY)

1. Transfer 300 μL of the fixed washed cells from the Indirect Staining Procedure or Direct Staining Procedure to a test tube. For indirect antibodies only, add one drop of Stabilizer 1 and vortex.
2. Centrifuge at 400 x g for 10 minutes and aspirate supernatant.
3. Resuspend cells in residual supernatant.
4. Place one drop of cells on a microscope slide. Cover with a 22 x 22 mm cover slip and seal with appropriate sealant if desired. Examine by fluorescence microscopy.

PROCEDURE - DIRECT STAINING

1. For each sample, label 12 x 75 mm test tubes for monoclonal antibody tests and appropriate isotypic controls. Add 200 μL of COULTER CLONE working dilution, 10 μL of CYTO-STAT/COULTER CLONE, or the appropriate amount of antibody as specified in the monoclonal antibody reagent package insert. Refer to specific package inserts for complete reagent information including cell count adjustment.
2. Pipette 100 μL of anticoagulated whole blood into the bottom of the properly labeled test tube. Be sure that the inside surface and top of the tube is free of blood. Vortex vigorously.
3. Incubate at room temperature for 45 minutes. (Incubations may be performed in an ice bath for enhanced fluorescence intensity.)
4. Wash two times with 4 mL of PBS, centrifuge at 400 x g for 3 minutes at room temperature, aspirate supernatant carefully. Vortex vigorously.
5. Add 1 mL of IMMUNO-LYSE working solution to each tube. Vortex vigorously.
6. Allow tubes to sit no less than 30 seconds, and no longer than 2 minutes before proceeding to the next step.
7. Add 250 μL of Fixative. Vortex immediately.
8. Wash two times with 3 mL of PBS, centrifuge at 400 x g for 3 minutes at room temperature, aspirate supernatant carefully. Vortex vigorously.
9. Add 1 mL of PBS and vortex.
10. Proceed according to the **Procedure-Fluorescence Microscopy** or **Flow Cytometric Results** heading.

QUALITY CONTROL

Monitor all samples for erythrocyte lysis by visual inspection of the sample tube after processing, and by evaluating light scatter histograms. Unlysed samples appear cloudy in the test tube. Partially lysed samples will contain heavy or aberrant leukocyte light scatter populations. Process and analyze a sample with known performance values with the unknown samples as a system control check.

For optimal results, ensure the COULTER EPICS XL/ XL-MCL, FC 500 flow cytometer or equivalent is properly aligned and standardized for light scatter and fluorescence intensity measurements and that color compensation is set if necessary. Refer to the flow cytometer product manuals for instructions.

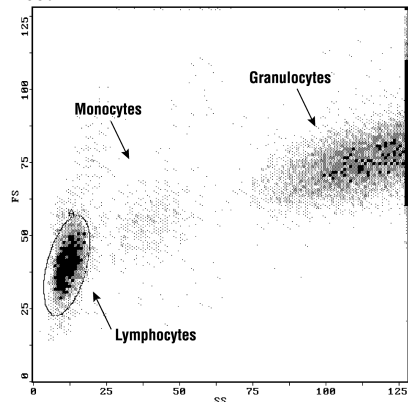
FLOW CYTOMETRIC RESULTS

NOTE: IMMUNO-LYSE frequently separates leukocytes into three discrete populations using a Side Scatter (SS) vs. Forward Scatter (FS) two-parameter histogram. Care must be taken to set the gate region to include the entire population being analyzed.

The representative data in Figure 1 was obtained using an EDTA anticoagulated normal whole blood specimen lysed with Whole Blood Lysing Reagents. Samples were

analyzed on a COULTER EPICS XL/XL-MCL flow cytometer using COULTER IsoFlow sheath fluid.

Figure 1. FS vs. SS of Lysed EDTA Normal Whole Blood



LIMITATIONS

1. Artfactual dual-staining may occur in some specimens stained with multiple monoclonal antibodies and lysed with whole blood lysing procedures.^{4,5,6}
2. Use reagents before the expiration date printed on the kit label.
3. Data is based upon normal whole blood samples.
4. Monitor all samples for erythrocyte lysis by visual inspection of the sample tube after processing, and by evaluating light scatter histograms. If samples appear cloudy, insufficient lysis has occurred.
5. Nucleated red cells (NRBC) may exhibit incomplete lysis and could be included in the leukocyte light scatter population.
6. For optimal results, ensure that the flow cytometer is properly aligned and standardized for light scatter and fluorescence intensity measurements and color compensation is set if necessary. Refer to the flow cytometer product manuals for instructions.

REFERENCES

1. Rose NR and Friedman H, eds. 1980. Manual of clinical immunology, 2nd edition. American Society of Microbiology, Washington, DC.
2. Guidelines for prophylaxis against pneumocystis carinii pneumonia for persons infected with human immunodeficiency virus. Center for Disease Control Morbidity and Mortality Weekly Report 38 (S-5):1-9.
3. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture (H3), Approved Edition. Clinical and Laboratory Standards Institute.
4. Nickolson JKA, Roa PE, Calvelli T, Stetler-Stevenson M, Browning SW, Yeung L, and Marti GE. 1994. Artfactual staining of monoclonal antibodies in two color combinations is due to an immunoglobulin in the serum and plasma. Cytometry (Communications in Clinical Cytometry) 18:140-146.
5. Ekong T, Gomples M, Clark C, Parkin J, and Pinching A. 1993. Double staining artifact observed in certain individuals during dual-color immunophenotyping of lymphocytes by flow cytometry. Cytometry 14:679-684.
6. Wilds D, Byrom N, Walker L, Habeshaw J and Dalgleish A. 1990. Characteristic Immunophenotyping artifact seen in patients with antimouse immunoglobulin antibodies. Cytometry 11:318-319.

PRODUCT AVAILABILITY

Whole Blood Lysing Reagents

[REF] 6602764 (100 tests)

For flow cytometry and fluorescence microscopy.

Whole Blood Lysing Reagents

[REF] 6603152 (300 tests)

For flow cytometry.

TRADEMARKS

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