




TRILLIUM DIAGNOSTICS, LLC

INNOVATIVE DIAGNOSTICS FOR CLINICAL CYTOMETRY AND LABORATORY HEMATOLOGY

Leuko64™

Assay for Detection of Inflammation and Tissue Injury

Product Information

 **LK-064-75 (75 tests) ; LK-064-250 (250 tests)**

 **For in vitro Diagnostic Use**

SUMMARY AND PRINCIPLE

Neutrophil CD64 expression is rapidly increased both *in vitro* and *in vivo* within hours by mediators of inflammation such as interferon-gamma and G-CSF (1-3). The same change is observed in response to documented infection or tissue injury, thus indicating the measurement of neutrophil CD64 expression correlates with the presence of such conditions in humans (4-12). The Leuko64 assay uses a mixture of three monoclonal antibodies with specificities to CD64 (clones 22 and 32.2) and CD163 (clone Mac2-158). The use of two antibodies to different epitopes of CD64 enhances the signal to noise ratio of the assay and provides a mechanism for minimizing lot-to-lot variation in the reagent fluorescence signal. The addition of the monoclonal antibody to CD163, a monocyte-specific antigen, increases the specificity of leukocyte subpopulation identification, thus facilitating the Leuko64 QuantiCALC™ software's cluster-finding algorithm and allowing for identification of internal "positive and negative control" populations. The use of automated software eliminates the variation inherent in the standard subjective flow cytometric listmode analysis. Using a fluorescence bead suspension for standardization of the cellular CD64 quantification allows traceability to the NIST Standard Reference Material SRM 1932 (RM 8640) and provides a mechanism to minimize lot-to-lot differences in the Leuko64 Assay kits. The results of the Leuko64 Assay are reported as a PMN CD64 Index as the diagnostic parameter, along with Monocyte CD64 and CD163 Indices.

APPLICATION

The LK-064-75 and LK-064-250 versions of the Leuko64 Assay kit are designed specifically for use with the following flow cytometers: Beckman Coulter XL, Beckman Coulter FC-500, BD Biosciences FACScan, BD Biosciences FACSCalibur, and BD Biosciences Cantos.

INTENDED USE

The Trillium Diagnostics Leuko64 assay kit is intended for use in the measurement of leukocyte neutrophil CD64 expression and intended for use in conjunction with other laboratory findings and clinical assessments to aid in the *in vitro* diagnosis of the acute systemic inflammatory response to tissue injury, such as occurs in tissue damage, infection, or sepsis. Trillium Leuko64 is intended for *in vitro* diagnostic use only by trained, qualified personnel.

KIT COMPONENTS

Reagent A - mixture of murine monoclonal antibodies (*contains buffered saline with 0.5% purified bovine serum albumin (BSA) and 0.01% sodium azide*)

Reagent B - 10X Concentrated Trillium Lyse solution (*contains ammonium chloride*)

Reagent C - suspension of 5.2 µm polystyrene beads labeled with StarFire Red and fluorescein isothiocyanate (FITC), (*contains < 0.1% sodium azide and 0.01% Tween 20*).

Leuko64 Software - Used to analyze flow cytometric listmode files and calculate CD64 Indices on leukocytes. The CD enclosed also contains tutorials for user training and optimal use of the Leuko64 assay kit.

REAGENTS and MATERIALS REQUIRED BUT NOT INCLUDED

Distilled Water

Flow Cytometer

Vortex Mixer

Disposable 12x75 polystyrene tubes

Micropipette(s) capable of dispensing 5µL, 50µL, and 1mL

Computer for listmode file analysis

SPECIMEN

The Leuko64 Assay requires only 50µL of anticoagulated whole blood. EDTA, heparin, sodium citrate, or ACD are all compatible with this test system. Blood specimens remain acceptable for up to 8 hours when held at room temperature (18-22°C) or for 48 hours when refrigerated (2-8°C).

SPECIMEN PREPARATION

- 1 Dilute the 10X Trillium Lyse (Reagent B) 1:10 by mixing 1 part of the concentrated Reagent B with 9 parts filtered distilled water. The final pH of this solution should be 7.40 ± 0.05 . Make a volume sufficient for anticipated number of tests (1.0 mL is required for each sample). Diluted or 1X Trillium Lyse is stable for 1 week at room temperature (20-26°C) or 30 days refrigerated (2-8°C).
- 2 Diluted Trillium Lyse solution must be between 20°C and 37°C when used. Cold solution may result in poor lysis and suboptimal assay conditions.
- 3 Label one 12 x 75 mm polystyrene tube for each sample to be analyzed.
- 4 Pipette 50µL of Leuko64 Reagent A into each labeled tube.
- 5 Pipette 50µL of the well-mixed anticoagulated blood sample with white blood count $< 25 \times 10^9$ cells/L (dilute as needed) to the corresponding tube containing Reagent A; gently mix or vortex; and incubate for 10 minutes in the dark at room temperature (18-22°C).
- 6 Add 1mL of 1X Trillium Lyse (diluted Reagent B) to each tube and thoroughly vortex. Incubate 15 minutes in the dark at room temperature. Intermittent vortexing enhances lysis.
- 7 Add 5µL Leuko64 beads (Reagent C) to each tube, vortex, and analyze on flow cytometer using instrument set-up and analysis protocol below. Prepared samples not immediately analyzed should be held at 2-8°C and shielded from light until analyzed. Analysis by flow cytometry should be performed within 4 hours of staining.

FLOW CYTOMETER SET-UP

Prior to analyzing your first specimen, an acquisition protocol will need to be set up on the flow cytometer. The Leuko64 beads (Reagent C) will serve as the calibrator for instrument gains and voltage settings. In order for the automated software to function properly, **it is imperative that the following steps be followed exactly**. See tutorials on the Leuko64 QuantiCALC software computer disc (CD) for additional instructional assistance.

- 1 Prepare a 12x75 polystyrene tube containing 5µL Leuko64 beads (Reagent C) in 0.5mL 1X Trillium Lyse (Reagent B diluted 1:10 with filtered distilled water, pH 7.40 ± 0.05). This bead suspension will be used to establish the optimal PMT voltage and light scatter settings of the flow cytometer.

- 2 In the acquisition mode of your cytometer (**Beckman Coulter XL or FC500 users- see below**), set up 5 two-parameter histograms:
- FS (lin) vs SS (log)
 - CD64 FITC vs SS (log)
 - CD163 PE vs SS (log)
 - FL3 (beads) vs SS (log)
 - CD163 PE vs CD64 FITC

And 3 one-parameter histograms:

- FL1 - CD64 FITC
- FL2 - CD163 PE
- FL3 (**or PMT with filter setup able to detect 685 nm light**)

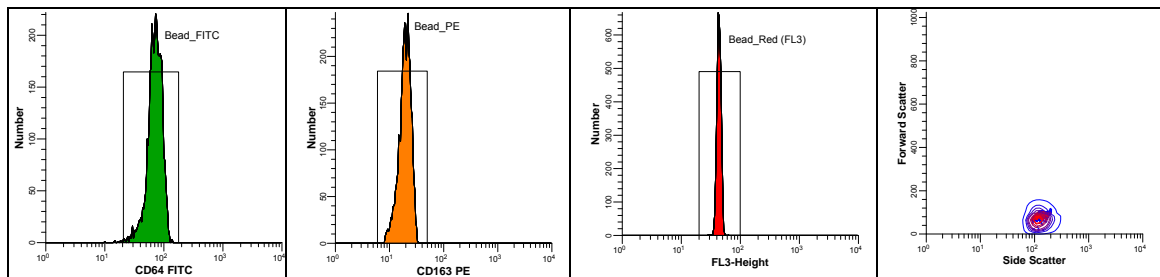
Note: Beckman Coulter XL and FC500 instruments **must** have the acquisition protocol parameters selected in the **following order**:

- P1 = FALS
- P2 = log side scatter
- P3 = log FITC
- P4 = log PE
- P5 = log PMT for 685 nm signal

- 3 Turn **OFF** all compensation and threshold settings.

- 4 Run the bead suspension and make the following adjustments on the one-parameter histograms (see diagram):

- The center of the peak on the FL1 (FITC) axis should be at the end of the second decade of fluorescence intensity.
- The center of the peak on the FL2 (PE) axis should be at ~20 (the first tic in the second decade of fluorescence intensity).
- The center of the peak on the FL3 axis should be mid-scale in second decade.



- 5 On the FS vs log SS histogram, the bead population should be positioned at the start of the third decade on log side scatter signal and at channel 85-100 on forward or low angle scatter signal (on 256 scale use channel 20-25).
- 6 The threshold to exclude platelets and red cell debris will be set on log SS using lymphocytes. The discriminator on log SS should be set just to the left of the lymphocyte population. Do not use a discriminator set on forward scatter, as beads will be excluded from acquisition.
- 7 Acquisition and Storage settings should be adjusted to allow for collection of 50,000 ungated events. Setting resolution at 1024 is generally recommended, but is **required** when using Beckman Coulter Cytomics™ FC500 or BD Biosciences FACSCanto™ cytometers.
- 8 Save the settings and acquisition template. Repeat steps 5-8 with any new lot of Leuko64 or following instrument service.

LISTMODE FILE ANALYSIS

Install enclosed Leuko64™ QuantiCALC software onto the Macintosh or PC that will be used for data analysis. Follow the user-friendly instructions for listmode data analysis within the Help file of the software. **NOTE: The software is lot-specific and protocols for use are instrument model-specific. You will be prompted to verify the proper selection.**



HANDLING AND STORAGE

Store vials upright, tightly capped, at 2-8°C when not in use. Unopened vials are stable until the expiration date indicated on each vial and assay sheet. Avoid unnecessary cycles of warming and cooling. Protect product from freezing, from temperatures above 30° C and from prolonged time at room temperature (18-26°C) or exposure to light.

WARNING

All components of this kit contain sodium azide (<0.1% w/ v). This chemical is a toxic and dangerous compound when combined with acids or metals. Handle with appropriate care. Solutions containing sodium azide should be disposed of properly.

PRODUCT QUALITY CONTROL

The performance and specificity of reagents contained in this kit are tested using Trillium's in-house quality control methods. Manufacturing of this product is done using quality system and manufacturing production guidelines in compliance with FDA QSR and ISO 13485:2003.

PRODUCT LIMITATIONS

- Therapeutic use of interferon-gamma, G-CSF or agents that modulate their levels are known to affect the up-regulation of leukocyte CD64 expression and therefore will affect the interpretation of the assay. Leuko64 Assay results should not be used as a sole measure of inflammation in these patients, but maybe used to monitor the effect of such treatments.
- Proper storage and use conditions of this product and the specimens to be tested must be adhered to for optimal performance and to avoid spurious or inaccurate results.
- Incomplete mixing of Reagent C prior to use may invalidate both the aliquot that is withdrawn and the remainder of the material in the vial. The selection of the incorrect instrument protocol or kit lot number when launching the software may result in a reduction in the accuracy of the listmode data analysis. The software is lot-specific and protocols for use are instrument model-specific. Make the proper selection when you are prompted to do so.
- Any file from a severely leukopenic or neutropenic blood sample showing less than 200 WBC events gated by the Leuko64 software in any of the 3 categories (PMN, Lymphocyte, or Monocyte) can not be analyzed by the Leuko64 assay without significant reduction in the accuracy and precision of the PMN CD64 index.
- Any file with a bead event count less than 1000 should be evaluated to determine the reason for the low bead count, which could be caused by improper instrument settings or an undiluted blood specimen having a WBC >25 x 10⁹ cells/L.
- Infrequently flow cytometry instruments may have filters arranged in an atypical configuration wherein FL1 is not the CD64 FITC signal and FL2 is not the CD163 PE signal and thereby compromising the performance of the Leuko64 software. Contact your instrument vendor and Trillium Diagnostics Technical Support for assistance in resolving this situation.

PERFORMANCE CHARACTERISTICS

Each laboratory should establish an acceptable reference range(s) for each lot of Leuko64™. The reference range of the PMN CD64 Index for healthy normal blood samples is anticipated to be ≤1.00. In general, the more severe the systemic acute inflammatory response, the higher the PMN (and Monocyte) CD64 Index value will be measured. The anticipated reference ranges for the Monocyte CD64 and CD163 Indices have not been established.

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TRADEMARKS

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