Leuko64™ Assay for Detection of Inflammation and Tissue Injury

Product Information

REF LK64-75 (75 tests) ; LK64-250 (250 tests)

IVD 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 (4-8). The Leuko64 assay uses a mixture of three monoclonal antibodies with specificities to CD64 clones 22 and 32.2 and CD163 Mac2-158, a RBC lysing solution, and a fluorescent bead suspension for instrument calibration and standardization of the leukocyte CD64 and CD163 expression in human blood.

APPLICATION

The LK64-75 and LK64-250 versions of the Leuko64 Assay kit are designed specifically for use with a flow cytometer.

INTENDED USE

The Leuko64 assay is intended for use in the measurement of leukocyte neutrophil CD64 levels, which increase in response to inflammation and tissue injury. Trillium Leuko64 is intended for *In Vitro* Diagnostic Use by trained, qualified personnel.

KIT COMPONENTS

Reagent A - mixture of murine monoclonal antibodies *(contains 0.1% sodium azide)*

Reagent B - 10X Concentrated Trillium Lyse solution *(contains ammonium chloride and purified bovine serum albumin)*

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.

REAGENTS and MATERIALS REQUIRED BUT NOT INCLUDED

Distilled Water
Flow Cytometer
Disposable 12x75 polystyrene tubes
Micropipette(s) capable of dispensing 5uL, 50uL, and 1mL
Vortex Mixer

SPECIMEN

The Leuko64 Assay requires only 50µL of anticoagulated whole blood. EDTA, heparin, sodium citrate, or ACD are all compatible with this test system. Specimens remain acceptable for up to 24 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. 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 x 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 should be held at 2-8°C, shielded from light until analyzed. Analysis should be performed within 6 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.**

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). This will be used to establish PMT voltage and scatter settings.
2. In the acquisition mode of your cytometer***(Beckman Coulter XL or FC500 users- see below)***, set up 4 two-parameter histograms:
   - FS (lin) vs SS (log)
   - CD64 FITC vs SS (log)
   - CD163 PE 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)***

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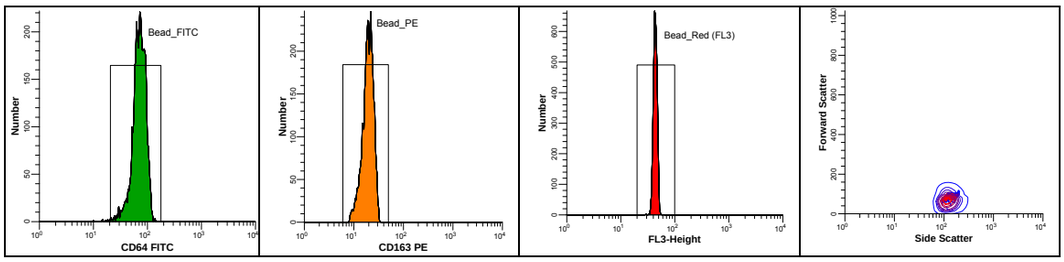
Beckman Coulter XL and FC500 instruments **must** have the acquisition protocol parameters selected in the **following order**:

- FALS
- log side scatter
- log, FITC
- log PE
- log PMT for 685 nM signal

3 Turn **OFF** all compensation 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.

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

Proper storage and use of this product as indicated above are required for optimal performance. Incomplete mixing of the Reagent C vial prior to use may invalidate both the aliquot that is withdrawn and the remainder of the material in the vial. The proper instrument protocol and kit lot number must be selected when launching the software for accurate listmode data analysis.

PERFORMANCE CHARACTERISTICS

Each laboratory should establish an acceptable reference range(s) for each lot of Leuko64™. The laboratory mean of the PMN CD64 Index for healthy normal blood samples is anticipated to be ≤1.00. The anticipated ranges for the Monocyte CD64 and CD163 Indices have not been established.

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