2. STATEMENT OF WARNINGS

3. Do not store the reagent in the refrigerator; do not freeze/thaw the tubes.
4. All blood samples must be considered as potentially infectious and must be handled with care. Protective gloves, gowns and goggles must be used while handling blood samples.
5. Discard reagent containing processed samples, as per applicable regulations, after sample acquisition and analysis.
6. Minimize the exposure of the tubes to light, especially during incubation of sample(s) stained with fluorescent antibodies or during processing of sample(s), before acquisition.
7. Only calibrated instruments, as per the manufacturer’s instructions, should be used.
8. Seal the zip lock of the pouch containing reagent tubes after removing the desired number of tests.
9. Reagent must be stored within the sealed pouch being exposed to moisture.

**STORAGE CONDITIONS**
Store the reagent between 20 and 30°C, in a dry place protected from direct exposure to light and moisture.

**EVIDENCE OF DETERIORATION**
Any damage to the reagent tube may indicate product deterioration and the product should not be used. Please contact your local distributor or you can contact Beckman Coulter at the following email address: duraclone-support@beckman.com.

**INSTRUMENT REQUIREMENTS**
This reagent is designed to be used on a flow cytometer such as Navios, capable of detecting forward and side scatter, and compatible with the emission spectra of the fluorochromes used in the reagent.

**SPECIMEN COLLECTION**
The whole blood sample should be collected in a blood collection tube containing K<sub>3</sub>EDTA. Follow the collection tube manufacturer’s guidelines for the minimum volume of blood to be collected. The sample must be stored between 18°C and 25°C. For other anticoagulants, it is recommended that the user verifies the reagent performance for their specific applications.

**MATERIAL REQUIRED BUT NOT SUPPLIED**
- Blood collection tube containing K<sub>3</sub>EDTA.
- Calibrated pipettes
- Vortex mixer
- Sheath fluid
- Flow-Check Pro Fluorospheres (REF. A63493) (For Navios alignment verification)
- Versalyse Lysing reagent (REF. A09777)
- Phosphate Buffered Saline (PBS) (1X)

**NOTE:** The sample preparation procedure mentioned in the section below are for reference purposes only. It may be necessary for users to adapt the protocol as per their specific requirements.

**PROCEDURE**
**SAMPLE PREPARATION FOR WHOLE BLOOD**
(Example)
1. Pipette 100 µL of whole blood into DuraClone IM Count Tube (Normal blood contains ~3000-11700 white blood cells per µL). Vortex at high speed for 6-8 seconds and incubate for 15 minutes at laboratory conditions (18-25°C).
2. Add 2 mL of VersaLyse, vortex on high for 1-3 seconds and incubate for 15 minutes at laboratory conditions (18-25°C).
3. The sample is ready for acquisition.
4. For sample acquisition on Navios/Gallios:
   a. Prepare an unstained sample by lysing 100µL of whole blood as per step(2) and use it to set the PMT voltages for FL1 and FL4 channels by setting the X-median of the unstained sample at 0.3 for each channel.
   b. Acquire the stained tube prepared using DuraClone IM Count Tube.

*Note: Keep the FL2 channel on and set PMT voltage at minimum setting.*
SAMPLE ANALYSIS (Figure 1: Example)

1. Create a CD45 FITC vs SSC dot plot. Create a Leukocytes gate to encompass all the CD45+ cells.
2. Create a 7AAD vs SSC gate and apply the Leukocytes gate.
   a. Create a gate to encompass the 7AAD+ cells: Dead.
   b. Create a gate to encompass the 7AAD- cells: Live.
3. Create a CD45 FITC vs PE dot plot. Create a Beads gate to encompass the events that show high fluorescence on FITC and PE.
4. Create a histogram of FITC vs Count and apply the Beads gate. Create a Bead singlets gate to encompass the singlets of beads and exclude the aggregates. Use the total number of Bead events, from the tube/pouch label or Certificate of analysis to calculate Cells/µL as described in the principle section. It is recommended to acquire 3000 bead events for estimating cell counts.

REFERENCES

PRODUCT AVAILABILITY
DuraClone IM Count Tube, 25 Tests, RUO

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*Navios is CE marked for 10-color in vitro diagnostic (IVD) use. In the U.S.A., Navios is intended for use as an IVD device for immunophenotyping with Navios tetra software and CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 reagents. All other uses are for research use only (RUO).

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