



Cell Suspension Workflow Guide

Mammalian White Blood Cells from Whole Blood PN# CEL-MWB1

Reagents Supplied by Sage Science			Storage Temp.
1 ea.	10X RBC Lysis Buffer, 275 ml	M1	4°C
1 ea.	Suspension Buffer, 30 ml	M2	4°C
1 ea.	Qubit Lysis Buffer, 25 ml	M3	RT
12 ea.	Cell strainers		

Materials Supplied or Prepared by User	Supplier	Cat#
Qubit™ Fluorometer and HS DNA Assay kit	ThermoFisher	Q32851
TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 to 8.0)	N/A	

Important!

- Whole blood should be collected with Acid Citrate Dextrose (ACD) or sodium EDTA anticoagulants, and stored at 4°C.
- Blood should be used within 5 days of collection.
- Maximum recommended cell load per lane for HLS cassettes contains **10 ug of genomic DNA**.
- Irrespective of input cell load, final input sample volume should be fixed at **70 ul per lane**.

CELMWB1 Workflow Guide Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
C1	D	12/12/17	9-2	Added Revision Change Log

Isolation of WBC: all steps at 4°C

(Dilute the provided 10X RBC Lysis Buffer (**M1**) in distilled H₂O to make 1X RBC Lysis Buffer. You will need 80ml of 1X RBC Lysis Buffer per cassette. Chill on ice before use.)

1. Mix the blood to ensure that it is a homogeneous solution. Add 12 mL whole blood to 37mL of cold 1X RBC lysis buffer.
2. Incubate for 5 minutes at 4°C. Halfway through this incubation gently invert tube 3 times to mix. The initial opaque, dark red solution will clear and become lighter in color.
3. Centrifuge at 2,400 x g for 4 minutes, decant and discard the supernatant.
4. Add 20mL 1X RBC lysis buffer.
5. Resuspend the cell pellet gently by pulsing 3 times for 2 to 5 seconds each, on a vortex set to 1800rpm. There should be no visible clumps left after vortexing.
6. Centrifuge at 2,200 x g for 2 minutes. Decant and discard the supernatant.
7. Repeat steps 4-6. The pellet should be almost completely clear of red or pink color.
8. After decanting the supernatant, let the tube sit for 1 minute, then aspirate the remaining buffer using a P1000 pipettor with 1ml tip.

Resuspension of WBCs in HLS Suspension Buffer buffer

1. Add 1mL of Sage HLS Suspension Buffer (**M2**) to the cells and resuspend them by slow, gentle pipetting with a P1000 pipettor. Note that the HLS Suspension Buffer solution is slightly more viscous than RBC lysis buffer due to a higher concentration of sucrose.
2. Examine the solution carefully for clumps, this is best done by drawing the solution into a 1mL pipet tip, and holding the tip up to the light.
3. If clumps are visible, filter the suspension through a 40 micron cell strainer (place the strainer in a new tube, and pour or pipet the cells into the strainer. Some tapping may be needed to start the liquid flow). Remove and discard the strainer.
4. Centrifuge for 10 to 20 seconds at 200 RPM to collect all the liquid at the bottom of the tube; the cells should not settle in this step.
5. Quantify the cells using a cell counter or a hemocytometer. Alternatively, determine the DNA content per unit volume of the suspension using the Qubit lysis procedure (Next Page). (The expected concentration of gDNA in the resuspended cell prep is 200 – 300 ng/ul.)

Quantification of DNA in a cell suspension using the Qubit HS assay

1. Gently mix the cells/spheroplasts by swirling, gentle vortexing, or pipetting. Transfer 10uL aliquots in triplicate to 1.5 ml microcentrifuge tubes.
2. To each microfuge tube add 190uL of Sage Qubit lysis buffer (**M3**) and mix by pipetting up and down several times. The mixing should be rapid, but should not generate bubbles or foaming - the goal is to disperse the cells quickly before they lyse and form a condensed clot of DNA.
3. Vortex the tubes as vigorously as possible for 30 – 60 seconds.
4. Add 600uL of TE (10 mM TrisHCl, 1mM EDTA, pH 7.5 to 8.0) to each tube, and vortex at maximum speed for 30-60 seconds.
5. If necessary, centrifuge (maximum speed) for 2 to 5 seconds to reduce any foam.
6. Dispense 5 ul of diluted lysed samples from step 5 into Qubit assay tubes.
7. Perform Qubit HS assay on the 5 ul sample from step 6. Use 195 of Qubit HS reagent for each lysed sample, and pipette Qubit assay mixture up and down several times to ensure homogeneity.
8. Read the Qubit assay tube concentration. Depending on the Qubit model, the Qubit tube concentration can be expressed as ng/ml or ng/ul (check!). Assuming the readout is ng/ml, DNA content of the original cell/spheroplast suspension can be calculated as:

$$[\text{Qubit tube conc, ng/ml}] \times (800/10) \times (200/5) = [\text{DNA conc original cell suspension, ng/ml}]$$

The first term is the dilution factor involved in making the diluted lysate (steps 2 and 4, above), and second term is the dilution factor for the Qubit HS assay (steps 6 and 7).

9. Average the three replicates to estimate the DNA content of the original suspension.
10. If replicates vary by >15%, the cells in the original suspension may be lysed or clumped.