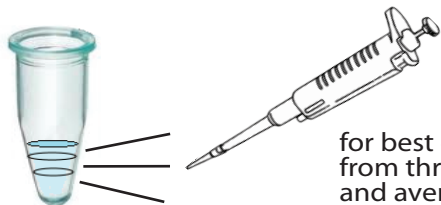


7. Prepare the Qubit HS reagent according to the manufacturer's protocol.
8. Add 195µl Qubit HS reagent to three Qubit assay tubes.
9. Transfer 5µl of the lysed cells to each of the Qubit tubes:



for best quantitation, pipette from from three depths of the sample and average the results

10. Take a triplicate Qubit measurement of each tube
11. Calculate the amount of DNA in the lysed cell sample:

Qubit reading in ng/ml x 0.200/5 (Qubit assay dilution factor) x 800/5 (cell suspension lysis dilution factor) = ng/µl (DNA content of the cell suspension)

D. Prepare WBC Suspension for loading on the SageHLS

1. Dilute the WBC Suspension with **Suspension Buffer (M2)** to prepare 10µg/70µl sample loads for the SageHLS.

sageHLS™

HMW Library System

Quick Guide

Mammalian White Blood Cell cell suspension kit

Product: CEL-MWB1
Revision B: June 20, 2017

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:

TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 to 8.0)
Qubit™ Fluorometer and HS DNA Assay kit

A. Whole Blood Collection

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.

B. Isolation of WBC from Blood: All Steps at 4°C

1. Dilute the **10X RBC Lysis Buffer (M1)** 1:10 (1 µl of 10X RBC in 9 µl of H₂O) to make 1X RBC Lysis Buffer. 80ml of 1X RBC Lysis Buffer will be required per cassette (2 samples). Chill on ice before use.
2. Mix the blood to ensure that it is a homogeneous solution. Add 12 mL whole blood to 37mL of cold 1X RBC lysis buffer.
3. Incubate for 5 minutes at 4°C. Halfway through the incubation, gently invert tube 3 times to mix. The initial opaque dark red solution will clear and become lighter in color.
4. Centrifuge at 2,400 x g for 4 minutes, decant and discard the supernatant.
5. Add 20mL 1X RBC lysis buffer
6. 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.
7. Centrifuge at 2,200 x g for 2 minutes, decant and discard the supernatant
8. Repeat steps 5 and 6. The pellet should be almost completely clear of red or pink color.
9. After decanting the supernatant, let the tube sit for 1 minute, then aspirate the remaining buffer using a P1000 pipettor with 1ml tip.

C. Resuspension of White Blood Cells in Suspension Buffer

1. Add 1mL of suspension buffer (**M2**) to the cells and resuspend them by mixing slowly with a P1000 Pipettor. Note that the suspension buffer (**M2**) solution is viscous.
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.

The expected concentration of gDNA in the resuspended cell prep is 200 – 300 ng/ul.)

C. DNA Quantitation of cell suspensions: Qubit Assay

1. Aliquot 5µl of the cell suspension into a 1.7 ml microfuge tube.
2. Add 195µl **Qubit Lysis Buffer (M3)** to the microfuge tube.
3. Mix the cells by pipetting up and down to thoroughly mix.
4. Vigorously mix the lysed cells.
5. Add 600µl of TE buffer to the lysed cells.
6. Vigorously mix again.