C. DNA Quantitiation of Lysed Spheroplasts: Qubit Assay

1. Aliqout 5µl of the WBC 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 thoroghly mix.
4. Vigorously mix the lysed cells.
5. Add 600µl of TE buffer to the lysed cells.
6. Vigorously mix again.
7. Prepare the Qubit HS reagent according 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:
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 (Spheroplast lysis dilution factor) = ng/µl (DNA content of the Spheroplast Suspension)

D. Prepare WBC Suspension for loading on the SageHLS

1. Dilute the WBC Suspension with **HLS Dilution Buffer** (**M4**) 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 A:	**March 27, 2017**

Reagents supplied by Sage Science:

1 ea.	10X RBC Lysis Buffer, 275 ml;	**M1**
1 ea.	Suspension Buffer, 30 ml;	**M2**
1 ea.	Qubit Lysis Buffer, 25 ml;	**M3**
1 ea.	HLS Dilution Buffer, 5 ml	**M4**
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

sage science
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460028 Rev A

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 (1µl 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 FSE to the cells and resuspend them by mixing slowly with a P1000 Pipettor. Note that the FSE 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 (next page).

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