Cell Suspension
Workflow Guide

Mammalian Tissue Culture Cells
PN# CEL-MWB1

Reagents Supplied by Sage Science			Storage Temp.
1 ea.	10X RBC Lysis Buffer, 275 ml (not used)	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#
Phosphate-buffered saline (PBS)	N/A	
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!

- Maximum recommended cell load per lane for HLS cassettes contains **10 ug of genomic DNA**, (approximately 1.5e06 human diploid cells).
- Irrespective of input cell load, final input sample volume should be fixed at **70 ul per lane**.

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Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
D	E	12/12/17	10-2	Added Revision Change Log

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

1. Obtain cells from culture flask, and wash at least 3 times by centrifugation and resuspension in phosphate-buffered saline (PBS) to remove media (centrifugation at 100-200Xg for 5-10 minutes, depending on cell type).

2. After third wash, resuspend cells in a small volume of PBS, and quantify cell concentration with cell counter or hemocytometer. Alternatively, determine the DNA content per unit volume of the suspension using the Qubit lysis procedure (next page).

3. Repellet the cells, and resuspend them in Suspension Buffer (**M2)** by gentle pipetting with a 1000ul pipette. The volume of HLS Suspension Buffer used should give a cell suspension containing less than or equal to 10 ug of genomic DNA per 70 ul (the loading volume for the HLS cassette). Note that the HLS Suspension Buffer solution is viscous.

   Note: While cell samples containing less than 10 ug of genomic DNA can be used, the loading volume should remain fixed at 70 ul for best DNA extraction results.

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:

   [Qubit tube conc, ng/ml] X (800/10) X (200/5) = [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.