HLS2
HMW Library System
sage science

Mammalian Tissue Culture
Cell Suspension and Preparation of Nuclei

PN# CEL-MTC1

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

Nuclear Isolation:

Reagents Supplied with this Kit			Storage Temp.
1 ea.	Nuclei Prep Buffer (NPB), 1000 ml	M4	4°C

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!

- Recommended input load of cells or nuclei should contain 5 µg of genomic DNA in a volume of 70ul. 5µg of human genomic DNA is equivalent to about 1.5 m diploid cells. Higher input loads will result in lower recovery and less target enrichment of CATCH targets.

CELMWB1 Workflow Guide
Mammalian Tissue Culture

Resuspend Cells in HLS Suspension Buffer

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.

STOP! If using cell suspensions skip to the Qubit Assay (page 4). Otherwise continue to the Nuclei preparation procedure, next page.

Preparation of Nuclei

Note: *This nuclei preparation procedure below works will with a wide range of cell inputs and buffer volumes. We have found that the same procedure can accommodate at least 5x more input cells, so adapt the volumes as needed.*

1. Resuspend WBC or cultured cells in cold Mg/Ca-free PBS (suggested volume of PBS, approx. 0.5 -1 mL for up to 1e09 mammalian cells).

2. To the concentrated cells in PBS, add 9 volumes of cold NPB (**M4**). Add NPB(**M4**) rapidly and smoothly disperse the cells by pipetting or low speed vortexing (60-70% of maximum speed). Add another 10 volume aliquot NPB (**M4**) to the tube and mix again thoroughly by inversion or low speed vortexing. (For example, starting with 1e08 cells in 1 mL of PBS, add 9 mL of cold NPB(**M4**), disperse by low speed vortexing. Then add 10 more mLs cold NPB (**M4**) and disperse again.)

3. Centrifuge for 1-2 min at 5,000 x g to pellet the nuclei.

CAUTION: Keep centrifugation time and speed to minimum to avoid irreversible aggregation of nuclei.

4. Remove the supernatant, flick the bottom of the tube to loosen the nuclear pellet. Resuspend the loosened pellet in 1-2ml NPB (**M4**). Pipette the nuclei thoroughly and smoothly using a regular orifice pipette tip (not wide-bore) to ensure complete resuspension. Add cold NPB (**M4**) to bring suspension to total volume used in step 2 (for the above example, 20 ml). Mix thoroughly by low speed vortexing. Recentrifuge, 1-2 minutes at 5,000 x g.

5. Remove the supernatant and resuspend the nuclei in NPB (**M4**) at a volume convenient for your experimental plan and quantify using the HLS rapid Qubit lysis method as described on the next page. Store at 4°C until ready for loading.

   NOTE: WBC and lymphoblastoid cell nuclei are very stable in NPB and we recommend loading nuclei into the HLS cassettes in NPB.

6. Dilute the nuclei with NPB (**M4**) to the desired concentration for your experiment. For instance, for CATCH we recommend input loads of nuclei containing 5-7 ug of total DNA in 70 ul of NPB (**M4**) (total DNA concentration 71-100ng/ul in 70 ul of NPB (**M4**)).

NOTE: The freezing protocol below works well for nuclei from lymphoblastoid cell lines, but does *NOT* work for nuclei from total WBCs generated by simple red cell lysis procedures.

7. **Freezing protocol for EBV immortalized lymphoblastoid cell lines:** Pellet cells from NPB by centrifugation 1-2 min. at 5000xg. Resuspend nuclei by vigorous pipetting in BAMBANKER serum-free freezing medium at a concentration of up to 2e07 nuclei per ml, and freeze in aliquots at -80C. (BAMBANKER is available in the US from Bulldog Bio, #BB01; in Europe, available from Nippon Genetics).

8. **Thawing protocol:** Frozen nuclei are thawed rapidly by stirring the frozen vials in a room temp water bath. After thawing, nuclei are diluted and thoroughly dispersed into 20 volumes of cold NPB (i.e.,1mL nuclei in BAMBANKER diluted into 20ml NPB), and collected by centrifugation 1-2 minutes, 5000xg. Nuclei are resuspended in a convenient volume of NPB and quantified by the Qubit rapid lysis procedure described in the HLS Cell Prep protocol.

NOTE: *Performance of frozen nuclei from lymphoblastoid cell lines such as GM24385(HG002) in CATCH is equivalent to freshly prepared nuclei.*

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

1. Gently mix the cells/spheroplasts/nuclei 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.

Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
D	E	12/12/17	5	Added Revision Change Log
E	F	10/13/22	1,3,4	Add NPB buffer to kit (1). Add Nuclei Prep Procedure (4)