HLS2
HMW Library System

sage science

Workflow Guide

Cell Suspension and Preparation of Nuclei from Mammalian WBC from Whole Blood

PN# CEL-MWB1

Isolation and Suspension of WBC:

Reagents Supplied with this Kit			Storage Temp.
1 ea.	10X RBC Lysis Buffer, 275 ml	**M1**	4ºC
1 ea.	HLS Suspension Buffer, 30 ml	**M2**	4ºC
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**	4ºC

DNA Quantification with Qubit

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!

- If possible, using cell nuclei with HLS-CATCH is recommended for highest reaction efficiency (higher enrichment and lower off-target background). Nuclei from lymphoblastoid cells are very stable and can be frozen before use. Cells should be fresh. Schedule availability of cells one day before using.

- 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.

    - 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.

Isolation of WBC: all steps at 4°C

(Dilute the provided 10X RBC Lysis Buffer (**M1**) in distilled H_20 to make 1X RBC Lysis Buffer. You will need 80 ml 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. The expected concentration of gDNA in the resuspended cell prep is 200 – 300 ng/ul.)

STOP! If using cell suspensions skip to the Qubit Assay (page 5). Otherwise continue to the Nuclei preparation procedure, next page.

Preparation of WBC 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^{o}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 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.

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Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
C1	E	12/12/17	5	Added Revision Change Log
E	F	9/15/22	1, 5	Add NPB buffer to kit (1). Add Nuclei Prep Procedure (4)