Fix cells for microC (human monocytes)

Date: 2023-05-25
Tags: fixation microC
Created by: Tsunghan Hsieh

Steps

Note: This protocol is modified for human monocytes (primary cells).

All steps before MNase digestion should be carried out in 15-ml tubes. The centrifuge should be carried out in swing-arm type instrument.

The collection and fixation should be carried out in medium containing serum, not PBS.

The below protocol is designed for 1E+6 cells. If there are more than 1E+6 cells, scale up all the reagents. Harvest cells from tissue culture plates in medium containing serum. Centrifuge and discard the supernatant. Store at -80C at least for 1 hour.

For per 1E+6 cells, prepare 0.3M DSG. Add 1mg of DSG into 10.22ul of DMSO.

Add DSG to 1ml medium containing serum.

Thaw 0.5M EGTA, 100mM MgCl2, 10X Nuclease Digest buffer at RT.

Turn heater to 37C. Thaw 10X Crosslink Reversal buffer and 20% SDS at 37C for 15min. Then put reagents back to RT.

Turn heater to 22C.

Thaw cells, wait 1min to let cells to reach RT. For per 1E+6 cells, suspend cells in 1ml medium containing 10ul of DSG.

Rotate cells for 10min at RT.

For per 1E+6 cells, add 27ul of 37% formaldehyde.

Rotate cells for 10mins at RT.

Centrifuge cells, 3000xg, 5min. Remove supernatant.

For 1E+6 cells, wash pellets with 200ul of 1X washing buffer. Centrifuge cells, 3000xg, 5min. Remove supernatant.

For one microC reaction, prepare 1X Nuclease Digest buffer. Add 5ul of 10X Nuclease Digest buffer and 5ul of 100mM MgCl2 into 40ul of ultra pure water. A 50-ul buffer can be used for 2-3E+6 cells.Store at

RT.

Resuspend 2-3E+6 cells in 50ul of 1X Nuclease Digest buffer. If more than 1E+6 cells are used, make each one reaction per 1E+6 cells.

Add 0.5ul of MNase Enzyme Mix. Pipet up and down for mixing.

Incubate the tube at 22C for exactly 15min in an agitating thermal mixer for 1250 rpm.

Stop the reaction by adding 5ul of EGTA. Pipet up and down for mixing.

Add 3ul of 20% SDS to lyse the cells. Pipet up and down for mixing.

Incubate 22C for 5min in an agitating thermal mixer for 1250 rpm.

After take 2.5ul of lysate, store other lysates into -80C.

Lysate QC.

Perform lysate QC by using Zymo Research RNA Clean & Concentrator - 5 kit.

Transfer 2.5ul Lysate to a new 1.5ml tube and labeled as QC tube.

Prepare the QC master mix by adding 5 ul of 10X Crosslink Reversal buffer and 1.5ul of Protease K into 45ul of ultra pure water.

Add 51.5ul of master to QC tube.

Incubate for the following program: 55C ,15min; 68C, 45min; 25C, hold.

Purify the QC sample using Zymo Research DNA Clean and Concentrator 5 -kit.

Add 200ul of DCC DNa binding buffer into QC tube and mix.

Centrifuge, 13000xg, 30sec. Discard the flow-through.

Add 200ul of DCC DNA Wash Buffer.

Centrifuge, 13000xg, 30sec. Discard the flow-through.

Repeat the wash step once.

Transfer the column onto a new 1.5-ml tube. Add 10ul of DCC Elute buffer. Stand at RT for 1min.

Centrifuge, 13000xg, 30sec. Collect the eluted DNA and run Bioanalyzer.

Unique eLabID: 20230525-3f6ea01862da03985cd0eb8bbe4b9167498d5484
Link: https://elabftw.science.ru.nl/database.php?mode=view&id=935