KAPP-Sen TMC: Fixation of Cells and Nuclei for Chromium Fixed RNA Profiling

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SEP 27, 2023

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.x54v9py5zg3e/v1

Protocol Citation: Juliana Alcoforado Diniz, Dylan Baker, Jessica Garofalo, Paul Robson 2023. KAPP-Sen TMC: Fixation of Cells and Nuclei for Chromium Fixed RNA Profiling. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9py5zg3e/v1

Protocol status: Working
We use this protocol and it's working

Created: Jul 28, 2023

Last Modified: Sep 27, 2023

PROTOCOL integer ID: 85646

Cellular Senescence Network (SenNet) Method Development Community

KAPP-Sen TM

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ABSTRACT

Cells are fixated prior to scRNA-seq according to 10X Genomics protocol CG000478.

Keywords: SenNet KAPP-Sen
TMC

Fixation of Cells & Nuclei

1 Centrifuge sample at 350 rcf for 5 min at 4°C.

2 Remove the supernatant without disturbing the pellet.

3 Add 1 ml Fixation Buffer to the sample pellet and pipette mix 5x.

4 Incubate for 1 h at room temperature (20°C) or for 16-24 h at 4°C for long term storage.

5 Centrifuge at 850 rcf for 5 min at room temperature.

6 Remove the supernatant without disturbing the pellet.

7 Add 1 ml chilled Quenching Buffer to the sample pellet and pipette mix 5x and keep on ice.

8 Determine cell concentration of the fixed sample using AO/PI (acridine orange/propidium iodide) Cell Viability Kit for Luna-FL automated cell counter.

9 Thaw Enhancer (**10x Genomics PN-2000482**) for 10 min at 65°C. Vortex and centrifuge briefly. Keep warm and verify no precipitate before use.

10 Add 1 volume pre-warmed Enhancer to fixed sample in Quenching Buffer. Pipette mix.

11 Store sample at 4°C for up to 1 week.

12 For long term storage (up to 6 months) also add 50% glycerol for a final concentration of 10% with Quenching buffer and store at -80°C.