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# Protocol for Nuclei/ Cell Isolation and 10X Genomics Fixed RNA profiling for Human Skeletal Muscle

Forked from [Protocol for Nuclei Isolation and Automated 10X Genomics Single Cell 5' Gene Expression for Human Ovary Explants](#)

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## DISCLAIMER

This protocol needs prior approval by the users' institutional review board (IRB) or equivalent ethics committee(s).

## ABSTRACT

This is the 10X Genomics protocol to fix, dissociate, and profile RNA from human skeletal muscle tissue.

## PROTOCOL REFERENCES

The following user guides from 10X Genomics were used for the different steps:

Tissue Fixation & Dissociation for Chromium Fixed RNA Profiling: *CG000553 Rev B*

Chromium Fixed RNA Profiling Reagent Kits for Multiplexed Samples: CG000527 Rev E

Chromium Fixed RNA Profiling Multiplexed Samples Pooling Workbook: CG000565 RevB

## GUIDELINES

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OPEN ACCESS



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[dx.doi.org/10.17504/protocols.io.14egn68z6l5d/v1](https://dx.doi.org/10.17504/protocols.io.14egn68z6l5d/v1)

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

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## Cell/Nuclei Isolation Protocol for Human Skeletal Muscle

- 1 The protocol CG000553 REV B was used to fix, dissociate, and isolate cells/nuclei from frozen human skeletal muscle with the following modifications:
  - 1) 1 mg / mL of Liberase TH was used for dissociation at Step 2b, Page 6.
  - 2) Two extra "spin only" (i.e., steps 3 and 4 ) of the Octodissociator protocol were run for each sample due to the presence of intact/ large tissue pieces at the end of the run at Step 2c, Page 6.
  - 3) Counts were performed using the Cellaca PLX Automated Cell counter (PN: PLX-SYS1) and ViaStain AOPI staining solution (PN: CS2-0106-5mL) at Step 2i on page 6.

<https://www.10xgenomics.com/support/single-cell-gene-expression-flex/documentation/steps/sample-prep/tissue-fixation-and-dissociation-for-chromium-single-cell-gene-expression-flex>

## Chromium Fixed RNA Profiling Reagent Kits

- 2 The protocol CG000527 Rev E was used to generate gene expression libraries from fixed cell/nuclei suspension inputs.

<https://www.10xgenomics.com/support/single-cell-gene-expression-flex/documentation/steps/library-prep/chromium-single-cell-gene-expression-flex-reagent-kits-for-multiplexed-samples>

Samples were multiplexed in batches of 16 using the user guide CG000565.

<https://www.10xgenomics.com/support/single-cell-gene-expression-flex/documentation/steps/library-prep/chromium-single-cell-gene-expression-flex-reagent-kits-for-multiplexed-samples>