

Oct 16, 2025

Human coronary nuclear isolation protocol

DOI

<https://dx.doi.org/10.17504/protocols.io.yxmvmmbqd5g3p/v1>

Daniel Yuhang Li¹, João Pinho Monteiro¹, João Pinho Monteiro, Daniel Yuhang Li

¹Stanford University



Quanyi Zhao

Stanford University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.yxmvmmbqd5g3p/v1>

Protocol Citation: Daniel Yuhang Li, João Pinho Monteiro, João Pinho Monteiro, Daniel Yuhang Li 2025. Human coronary nuclear isolation protocol. protocols.io <https://dx.doi.org/10.17504/protocols.io.yxmvmmbqd5g3p/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: October 15, 2025



Last Modified: October 16, 2025

Protocol Integer ID: 229812

Keywords: human coronary nuclear isolation protocol, human coronary nuclear isolation protocol this protocol, quality nuclei from human coronary tissue, clean nuclei suitable for fac, preservation of nuclear integrity, yielding clean nuclei, human coronary tissue, nuclear integrity, quality nuclei, 10x genomics capture, nucleus application

Abstract

This protocol outlines a streamlined method for isolating high-quality nuclei from human coronary tissue for downstream single-nucleus applications. The approach ensures efficient lysis, removal of debris, and preservation of nuclear integrity, yielding clean nuclei suitable for FACS and 10x Genomics capture.

Buffers

- 1 Nuclear buffer (Premade and stored at 4C)
 - 500mL H₂O
 - 1mL NaCl (5M) for final concentration 10mM
 - 1.5mL MgCl (1M) for final concentration 3mM
 - 5mL Tris-HCl (1M) for final concentration 10mM
- 2 10% BSA
 - 10% BSA solution (Sigma-Aldrich, A1595-50ML)
- 3 Lysis buffer (1mL)
 - 1uL NP-40 (1:1000) for final concentration 0.1%
 - 10uL 10% BSA (1:100)
 - 2uL 5% Digitonin (1:1000)
 - 1uL Spermidine (500 mM stock) for final concentration 0.5mM
 - 1uL Spermine (150 mM stock) for final concentration 0.15mM
 - 25uL Protector RNase Inhibitor (40 U/uL stock) for final concentration 1 U/uL
- 4 Wash buffer (1mL)
 - 975uL Nuclei buffer
 - 10uL 10% BSA
 - 25uL Protector RNase Inhibitor (40 U/uL stock) for final concentration 1 U/uL
- 5 1x nuclei buffer (1mL)
 - 950uL H₂O
 - 10uL 10% BSA
 - 50uL 20x nuclei buffer from 10x kit

Protocol

- 6 Cut a 30-40mg piece of tissue on a clean metal block cooled to freezing temp over dry ice. Keep sectioned and weighed tissue in 1.5ml Lo Bind tube on dry ice until ready to proceed.
NOTE: Large pieces of tissue >60mg will likely be underlysed and block the filters.
- 7 Place on ice, add 500uL of lysis buffer and finely mince tissue with small iris scissors (4 min).
Timing can be adjusted depending on tissue size, for 30-40mg this was sufficient.
- 8 Gently vortex and rest on ice for 5 min with intermittent vortexing.
- 9 Stop excess lysis by diluting in 500uL of wash buffer and proceed with filtering steps.



- 10 Filter through 70um strainer into new 1.5uL Lo Bind tube coated with BSA.
May need gentle centrifugation to move lysate through filter.
- 11 Repeat filtering through 10um strainer into new 1.5uL Lo Bind tube coated with BSA.
- 12 Discard strainer and add 500uL of wash buffer to further dilute detergent.
- 13 Centrifuge nuclei at 500xg for 10min at 4C
- 14 Remove supernatant and add 150uL of "10x nuclei buffer" without disturbing the pellet.
- 15 Centrifuge nuclei at 500xg for 5min at 4C and remove all supernatant (buffer exchange)
- 16 Resuspend in 7-30uL of 1x nuclei buffer.
- 17 FACS sorting prior to capture aim for at least 200k nuclei total and resuspend
in ~500uL of chilled Wash Buffer (w/ BSA).
- 18 Assess nuclei quality and count with Trypan blue.
- 19 Proceed to 10X capture.