

# Single-cell isolation from embryonic chicken heart



*Developed by Iwijn De Vlamincck and Jonathan T. Butcher*

## About the De Vlamincck and Butcher labs

The Iwijn De Vlamincck lab uses spatiotemporal single cell RNA sequencing to investigate questions related to health and development. The lab specializes in work on infectious and immune-mediated diseases, as well as spatial profiling of microbiomes and infection in complex tissues.

The Jonathan T. Butcher lab studies how mechanical forces shape the developing heart and understanding how to apply these principles to human health. Their main focuses within development are on cardiovascular developmental biomechanics, and within health they study reactivation of developmental biology in valve diseases as well as tissue engineering and regeneration.

### Notice

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**This protocol was used in**

Spatiotemporal Atlas of Heart Development Reveals Blood-Flow-Dependent Cellular, Structural, Metabolic, and Spatial Remodeling. Jooyoung Park, Shuofei Sun, Rohit Agarwal, Andreas Stephanou, Mong Lung Steven Poon, Hyun Maeng, Peyton Lancaster, Iwijn De Vlamincq, Jonathan Butcher

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## Materials

Material	Supplier	Part Number	Notes
Centrifuge with swinging bucket rotor	Varies	Varies	Compatible with 15 mL or 5 mL centrifuge tubes and capable of reaching 4°C.
Shaker	Varies	Varies	Compatible with 1.5mL tubes
1.5 mL Protein LoBind® Tubes	Eppendorf®	022431081	Or equivalent
40 µm cell strainer	Corning®	431750	Or equivalent
Collagenase, Type II, powder	Thermo Fisher Scientific®	17101015	
Hanks' Balanced Salt solution	Merck®	H9394	Or equivalent
RNaseZAP® RNase Decontamination Solution	Thermo Fisher Scientific	AM9780	

## Preparation

1. Clean the bench top and dissection area with 70% ethanol, followed by RNaseZAP to remove RNases.

2. Prepare the following solutions, and add reagents in the order provided in the table.

### Reconstituted collagenase II stock example



**Note:** Each lot will have a different value of U/mg, so make note of the final concentration when reconstituted in 1mL

Reagents	Stock Concentration	Final Concentration	Total amounts
Collagenase II powder	265 U/mg	265 U/ $\mu$ L	1 g
Hank's Balanced Salt Solution	N/A	N/A	1 mL

### Working concentration collagenase II example



**Note:** Each lot will have a different value of U/mg, so adjust according to the true concentration of your stock.

Reagents	Stock Concentration	Final Concentration	Total amount for 1 mL
Reconstituted collagenase II stock	265 U/ $\mu$ L	300 U/mL	1.13 $\mu$ L
Hank's Balanced Salt Solution	N/A	N/A	1 mL

## Procedure

1. Dissect hearts and place in ice-cold Hank's Balanced Salt Solution
2. For hearts from embryos 7 days old or less, place the hearts directly in 500  $\mu$ L of 300 U/mL collagenase type II in a 1.5 mL tube and incubate for 15 minutes at 37°C on a shaker. For hearts of embryos older than 7 days, chop hearts into small pieces using a razor blade and then transfer into 1 mL of 300 U/mL collagenase type II in a 1.5mL tube and incubate for 20 minutes at 37°C on a shaker.
3. Centrifuge and remove supernatant



**Note:** Centrifugation conditions may vary between instruments. Users should ensure that all centrifugation steps result in cells suitable for analysis before proceeding with fixation and barcoding.

4. For embryos 7 days old or younger, resuspend cells in 500  $\mu$ L of collagenase II and incubate for 10 minutes at 37°C on a shaker. For embryos older than 7 days, resuspend cells in 500  $\mu$ L of collagenase II and incubate for 10 minutes at 37°C on a shaker.
5. Pass cells through a 40  $\mu$ m strainer into a new 1.5 mL tube
6. Centrifuge and resuspend in PBS



**Note:** Red blood cell depletion may be required. It is recommended to assess the fraction of red blood cells before proceeding.

7. Count cells and assess quality



**Note:** Accurate counting and assessment of cells is critical to generate high-quality data. If using a hemocytometer, use all four quadrants for accuracy. View at 10, 20, and 40X for morphology and debris.

Optimally, less than 5% aggregation and 5% debris. A best practices resource for hemocytometer use, see:

<https://www.hemocytometer.org/>

8. Proceed to the appropriate Parse Biosciences Cell Fixation Kit [User Guide](#) (either standard Cell Fixation, Low-Input Fixation).

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