

Nov 25, 2025

Single-cell dissociation from zebrafish larvae

DOI

<https://dx.doi.org/10.17504/protocols.io.bp2l6zqn5gqe/v1>

Stephan Schreiber¹

¹Helmholtz Centre for Environmental Research GmbH - UFZ



Stephan Schreiber

Helmholtz Centre for Environmental Research GmbH - UFZ

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.bp2l6zqn5gqe/v1>

Protocol Citation: Stephan Schreiber 2025. Single-cell dissociation from zebrafish larvae. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bp2l6zqn5gqe/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: November 24, 2025

Last Modified: November 25, 2025

Protocol Integer ID: 233348

Keywords: zebrafish, single-cell, dissociation, cell suspensions from zebrafish larvae, cell dissociation from zebrafish larvae, cell sequencing, zebrafish larvae, cell transcriptomic analysis, 10x genomics chromium platform, cell analysis, such as the 10x genomics chromium platform, cell viability assessment, cell viability, transcriptomic analysis, cell, cell suspension, cell resolution, cell dissociation

Disclaimer

This protocol is provided as a detailed description of the procedures used in our laboratory. While every effort has been made to ensure accuracy and reproducibility, users must adapt the protocol to their own experimental conditions, equipment, and institutional guidelines. The authors assume no responsibility for any outcomes resulting from the use of this protocol. All procedures involving animals or animal-derived material must comply with the user's local regulations and require approval by the appropriate ethics committee where applicable.

Abstract

This protocol describes a robust and reproducible method for generating single-cell suspensions from zebrafish larvae (96 and 120 hpf) for downstream single-cell analysis, such as the 10X Genomics Chromium platform. The procedure includes enzymatic digestion using TrypLE and Collagenase/Dispase in a supplemented HBSS buffer, followed by mechanical dissociation and multiple filtration and washing steps. Critical quality control steps ensure cell viability (>80%) and concentration (>400 cells/ μ L), which are essential for high-quality single-cell sequencing. This SOP emphasizes the use of standardized reagents, precise timing, and temperature-controlled equipment to ensure consistency across samples. The workflow is optimized for processing up to three samples simultaneously and includes detailed buffer compositions, cell viability assessment using Trypan Blue, and guidelines for chip loading. This method enables high-throughput and reproducible single-cell transcriptomic analysis from whole zebrafish larvae, facilitating developmental and toxicological studies at single-cell resolution.

Attachments



LTF_SOP_22_Single-

ce...

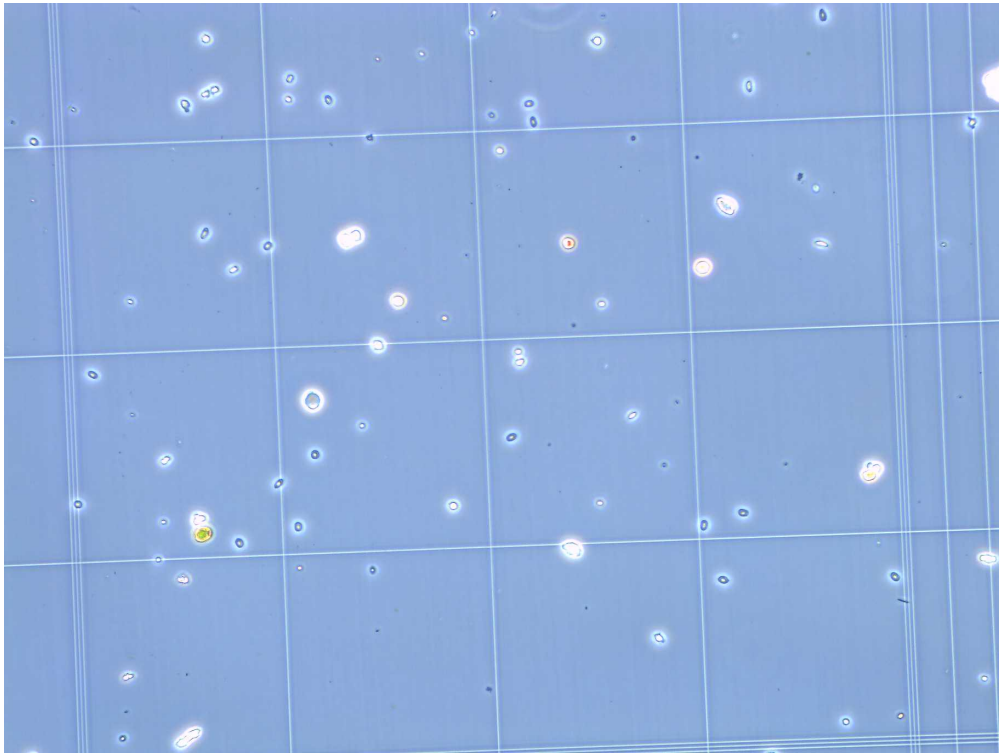
14KB

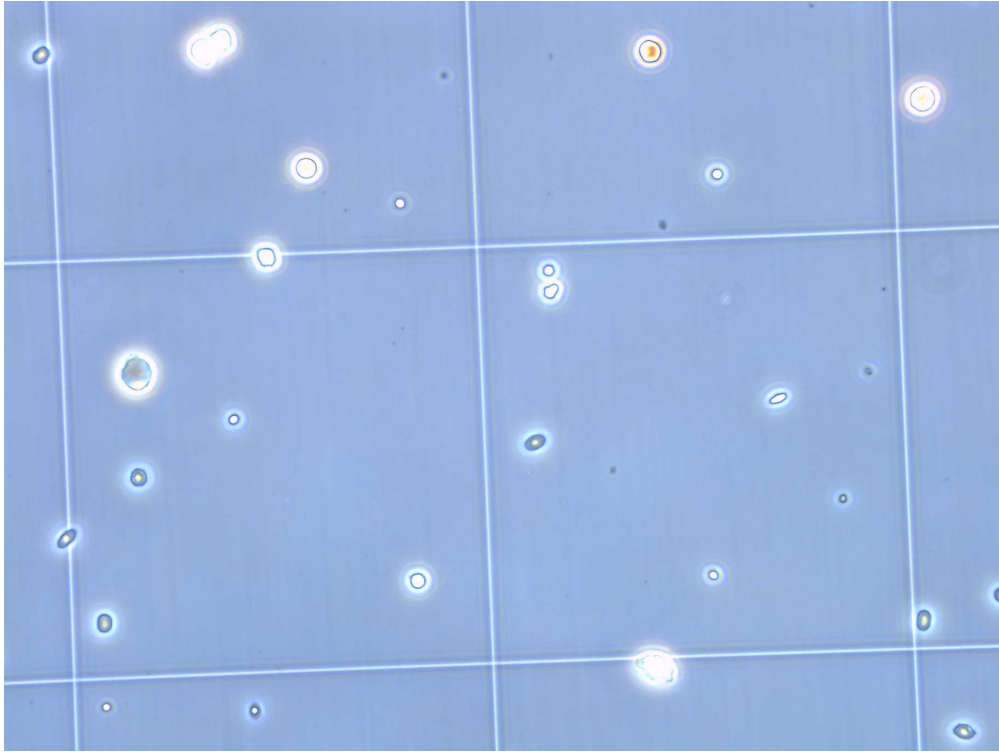


SOP_22_v5_Single-cel...

180KB

Image Attribution





Guidelines

Sample Handling

- Process no more than three samples at a time to maintain precise timing and handling consistency.
- Use fresh larvae from a 28 °C incubator; do not chill on ice prior to dissociation.

Sterility and Preparation

- Disinfect the biological safety cabinet before and after each session.
- Pre-cool the centrifuge to 4 °C and pre-warm the Thermomixer to 28 °C in advance.

Enzyme & Buffer Use

- Prepare buffers fresh where indicated and keep them at appropriate temperatures (on ice or 28 °C).
- Use single-use aliquots of Collagenase/Dispase; never refreeze thawed aliquots.

Pipetting Techniques

- Use wide-bore pipette tips during cell resuspension to avoid damaging cells.
- Pipette gently and consistently to support mechanical dissociation without excessive shear stress.

Filtration Protocol

- Perform two filtration steps: first with a 70 µm, then with a 40 µm FlowMi pipette tip filter, to remove aggregates and tissue debris.

Quality Control

- Ensure cell viability >80% and concentration >400 cells/µL prior to chip loading (e.g., 10X Genomics).
- Use the Neubauer chamber and Trypan Blue staining for viability and cell counting assessments.

Documentation

- Complete the LabTrackingForm with all relevant data, including cell counts, viability, and any irregularities.

Materials

- Collagenase/Dispase (Roche, 10269638001)
- TrypLE (Thermofisher, A1217701)
- HBSS (Gibco, 14175-095)
- HEPES buffer (Sigma, H0887)
- DMEM/F-12 (Gibco, 11039021)
- Ultrapure BSA (Ambion, AM2618)
- Calf serum (Gibco, A31605-01)
- 1.5 mL LowBind SafeLockTubes – PCR clean (Eppendorf, 0030108051)
- Trypan-Blue (Sigma-Aldrich, T8154-20ML)
- 70 µm FlowMi pipette tip cell strainer (Sigma-Aldrich, BAH136800070)
- 40 µm FlowMi pipette tip cell strainer (Sigma-Aldrich, BAH136800040)
- 1250 µL pipette filter tip (Eppendorf, 0030078594)
- 300 µL pipette filter tip (Eppendorf, 0030078560)
- 100 µL pipette filter tip (Eppendorf, 0030078543)
- 10 µL pipette filter tip (Labsolute, 7695880)
- 200 µL ART wide bore pipette filter tip (Fisher Scientific, 2069GPK)
- 1000 µL ART wide bore pipette filter tip (Fisher Scientific, 2079GPK)

Required equipment:

- Biological safety cabinet
- Multifuge X1R
- Eppendorf Thermomixer for 1.5 mL tubes
- Eppendorf Research Plus pipettes (10 µL, 100 µL, 200 µL, 1000 µL)
- Timer
- Neubauer counting chamber

Buffers:

- Washing buffer:

- DMEM/F-12: 3968 µL (4× 992 µL)
- BSA: 0.04%, 32 µL

- Quenching buffer:

- DMEM/F-12: 1784 µL (2× 892 µL)
- Calf serum: 10%, 200 µL
- BSA: 0.04%, 16 µL

- Dissociation buffer:

- Suppl. HBSS: 1744 µL (2× 872 µL)
- TrypLE: 1X, 200 µL
- Collagenase/Dispase: 2 mg/mL, 40 µL
- BSA: 0.04%, 16 µL

Safety warnings

Enzyme Inactivation Risk

- Collagenase/Dispase loses activity after thawing; using refrozen aliquots can lead to incomplete dissociation and poor yield.

Timing Sensitivity

- Over- or under-incubation during enzymatic digestion affects both viability and dissociation efficiency. Strictly adhere to the timing protocol (e.g., 3-minute intervals ×5).

Cross-contamination

- Failure to work under sterile conditions or neglecting equipment cleaning can lead to contamination, jeopardizing single-cell workflows.

Shear Damage

- Overly vigorous pipetting can compromise cell membrane integrity, reducing viability and introducing bias in downstream single-cell data.

Filter Blockage

- Skipping filtration or using clogged filters can result in clumped cells, which may block microfluidic channels during 10X chip loading.

Insufficient Cell Quality

- Proceeding to chip loading with poor-quality suspensions (viability <80%, low concentration) will likely lead to sequencing failure or data dropout.

Improper Disposal

- Improper waste management of reagents and biological material may violate biosafety regulations. Always follow local disposal protocols

Ethics statement

This protocol involves the handling and dissociation of zebrafish (*Danio rerio*) larvae. Depending on national or regional regulations, zebrafish may be considered protected vertebrate animals at or after certain developmental stages (commonly around 72 hours post-fertilization). Therefore, users must ensure that all procedures described in this protocol comply with their local legislation and institutional requirements.

Prior approval from the users' Institutional Animal Care and Use Committee (IACUC), Animal Welfare Body, or an equivalent ethics committee may be required before conducting work based on this protocol. Users are responsible for obtaining all necessary permissions and for adhering to relevant national and international regulations governing the use of animals for scientific purposes (e.g., EU Directive 2010/63/EU or corresponding local frameworks).

The authors performed experiments in compliance with applicable regulations and under valid institutional approval. However, requirements may differ between countries or institutions, and users must verify and follow the rules that apply to their own research environment.

Before start

- More than 3 samples are difficult to handle and not recommended.
- Always use fresh larvae from 28°C incubator
- For 96 hpf and 120 hpf larvae, pool 5 individuals per sample

Before you start:

- The working surface of the safety cabinet must be disinfected
- Pre-cool centrifuge to 4°C
- Pre-warm Thermomixer to 28°C
- Wear a lab coat and gloves for all working steps
- Prepare a LabTrackingForm to note cell preparation information



Reagent and Buffer Preparation

1 Collagenase/Dispase

Prepare in advance. Resuspend the Collagenase/Dispase in nuclease-free water to 100mg/mL. Prepare single-use aliquots (45 μ L) and store them at -20 $^{\circ}$ C or -80 $^{\circ}$ C . Once thawed, do not refreeze/reuse.

2 Supplemented HBSS

Prepare in advance, store at 4 $^{\circ}$ C , and warm to 28 $^{\circ}$ C before the start of the experiment.

	A	B	C
	Ingredient	Final concentration	Amount
	HBSS	-	49225 μ L
	CaCl ₂	0.5 nM	25 μ L
	HEPES	15 mM	750 μ L

3 Dilution buffer

Prepare fresh and keep On ice until use.

	A	B	C
	Ingredient	Final concentration	Amount
	HBSS (unsuppl.)	-	496 μ L
	BSA	0.04%	4 μ L

4 Washing buffer

Prepare fresh and keep On ice until use.

	A	B	C
	Ingredient	Final concentration	Amount



	A	B	C
	DMEM/F-12	-	3968 μ L (4 $\times$ 992 μ L)
	BSA	0.04%	32 μ L

5 Quenching buffer

Prepare fresh and keep at  28 °C until use.

	A	B	C
	Ingredient	Final concentration	Amount
	DMEM/F-12	-	1784 μ L (2 $\times$ 892 μ L)
	Calf serum	10%	200 μ L
	BSA	0.04%	16 μ L

- 6 Prepare fresh and keep at  28 °C until use. Add Collagenase/Dispase just directly before usage.

	A	B	C
	Ingredient	Final concentration	Amount
	Suppl. HBSS	-	1744 μ L (2 $\times$ 872 μ L)
	TrypLE	1X	200 μ L
	Collagenase/Dispase	2 mg/mL	40 μ L
	BSA	0.04%	16 μ L

Collection of zebrafish larvae

- 7 Perform the following working steps at  Room temperature :



- 7.1 Obtain zebrafish larvae from a  28 °C incubator (do not place larvae on ice!).
- 7.2 Per sample, transfer five larvae into fresh 1.5 mL LowBind SafeLockTubes with some cultivation water.
- 7.3 Place tubes with collected larvae in the Thermomixer at  28 °C .

Wash zebrafish larvae

- 8 Perform the following working steps under a safety cabinet:
 - 8.1 Carefully take off water without pipetting the larvae and immediately add  1 mL of supplemented and pre-warmed HBSS.
 - 8.2 Carefully take off  900 µL HBSS without pipetting the larvae.
 - 8.3 Add TrypL and Collagenase/Dispase to Dissociation-Mix.
 - 8.4 Carefully take off the rest of HBSS without pipetting the larvae using a 300 µL pipette tip.

Dissociate zebrafish larvae

- 9 Perform the following working steps under a safety cabinet:
 - 9.1 Immediately add  500 µL of prewarmed Dissociation buffer.
 - 9.2 Incubate larvae at  28 °C for  00:03:00 .
 - 9.3 Using a 1 mL tip, pipette up and down the larvae 20 times.



9.4 Incubate larvae at 28 °C 28 °C for 00:03:00 .

9.5 Using a 1 mL tip, carefully pipette up and down the larvae 15 times.

9.6 Repeat the pipetting/incubation step every 3 minutes for 5 cycles (15 minutes).

9.7 Add 500 μ L quenching buffer per dissociated ZFE (per tube), and mix by careful pipetting using a normal 1 mL tip 10 times.

Wash cells

10 Perform the following working steps under a safety cabinet:

10.1 Immediately strain the cells carefully through a 70 μ M FlowMi pipette tip cell strainer into a fresh 1.5 mL LowBind SafeLockTube.

10.2 0 μ L Spin down cells for 300 x g, 4°C, 00:05:00 (acceleration set to 7 and brake to 8) and place on ice.

10.3 Carefully take off the supernatant without disturbing the pellet.

10.4 Add 1 mL of prechilled washing buffer to the cell pellet.

10.5 Carefully resuspend cells by pipetting them up and down using a wide-bore pipette tip and placing them on ice.

10.6 Immediately strain the cells carefully through a 40 μ M FlowMi pipette tip cell strainer into a fresh 1.5 mL LowBind SafeLockTube.

- 10.7 Spin down cells for  300 x g, 4°C, 00:05:00 (acceleration set to 7 and brake to 8) and place on ice.
- 10.8 Carefully take off the supernatant without disturbing the pellet.
- 10.9 Add  100 µL of prechilled dilution buffer to the cell pellet (total volume ~ 120 µL).
- 10.10 Carefully resuspend cells by pipetting them up and down using a wide-bore pipette tip and placing them on ice.
- 10.11 Start cell counting and cell viability estimation.

Cell viability determination

- 11 Perform the following working steps under a safety cabinet:
 - 11.1 Transfer  10 µL Trypan-Blue into a fresh 1.5 mL LowBind SafeLockTube.
 - 11.2 Add  10 µL of cell suspension to Trypan-Blue.
 - 11.3 Mix by carefully flicking.
 - 11.4 Apply 10 µL of the stained cell suspension to a Neubauer counting chamber.
 - 11.5 In four quadrants, count alive and dead cells separately and note the results in the LabTrackingForm.
 - 11.6 Take a picture using the microscope camera for documentation.
 - 11.7 Calculate the viability rate.

Cell counting

- 12 Perform the following working steps under a safety cabinet:
 - 12.1 Apply 10 μ L of the unstained cell suspension to a Neubauer counting chamber.
 - 12.2 Count all cells in four quadrants and note the LabTrackingForm results.
 - 12.3 Take a picture using the microscope camera for documentation.
 - 12.4 Calculate the cell number per μ L.

Quality control

- 13 Visually check the cell suspension using a microscope.
 - 13.1 Visually check the cell suspension for undissociated tissue and debris. It should be as clean as possible.
 - 13.2 Check if the cell concentration is sufficient for the 10X single-cell workflow (> 400 cells per μ L).
 - 13.3 Check if cell viability is sufficient for the 10X single-cell workflow (> 80%).

Chip loading

- 14 Perform the following working steps in a dust-free environment.
 - 14.1 Prepare the 10X Chip and MasterMix according to the manufacturer's instructions.



14.2 Strain the cell suspension (~100 μ L) carefully through a 40 μ M FlowMi pipette tip cell strainer into a fresh 1.5 mL LowBind SafeLockTube.

14.3 Add cell suspension to MasterMix and start Chip loading.

Completion

15 Cleaning

15.1 Clean all used materials and return them to their original place.

15.2 Clean and disinfect the safety cabinet.

15.3 Turn off all used instruments.

Protocol references

1. Literature references on zebrafish larvae and cell isolation

- Dylan R. Farnsworth, Lauren M. Saunders, Adam C. Miller - *A single-cell transcriptome atlas for zebrafish development*

- Erica Bresciani, Elizabeth Broadbridge, Paul P. Liu - *An efficient dissociation protocol for generation of single cell suspension from zebrafish embryos and larvae*

2. 10X Genomics documents

- 10X Genomics - *Single Cell Protocols – Cell Preparation Guide* • Rev C (CG00053)

- 10X Genomics - *Cell Preparation for Single Cell Protocols* • Rev D (CG00053)



Acknowledgements

I would like to thank all colleagues who supported me during the establishment and optimization of this protocol. Special thanks go to **Tim Jonat**, **Nicole Schweiger**, **Janet Krüger**, and **Dr. Tamara Tal** for their valuable advice, scientific discussions, and for providing zebrafish samples and related materials. I also gratefully acknowledge the support of external collaborators and scientific partners, including **Dr. Yavor Hadzhiev** (University of Birmingham - Institute of Cancer and Genomic Sciences), who contributed with his expertise and constructive feedback throughout the development process.