

## Workflow SOP 22.5

### Single-cell dissociation from zebrafish larvae

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#### Document History

Version	Date	Author	Changes
22.1	21.02.2024	Stephan Schreiber	Initial revision
22.2	31.01.2025	Stephan Schreiber	Updated cell handling description
22.3	28.04.2025	Stephan Schreiber	Minor changes and corrections
22.4	02.06.2025	Stephan Schreiber	2 <sup>nd</sup> 40 µM filter step added
22.5	14.07.2025	Stephan Schreiber	Last 40 µM filter step before chip loading



QR-code for SOP-Name

**Required material:**

- 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  $\mu$ m FlowMi pipette tip cell strainer (Sigma-Aldrich, BAH136800070)
- 40  $\mu$ m FlowMi pipette tip cell strainer (Sigma-Aldrich, BAH136800040)
- 1250  $\mu$ L pipette filter tip (Eppendorf, 0030078594)
- 300  $\mu$ L pipette filter tip (Eppendorf, 0030078560)
- 100  $\mu$ L pipette filter tip (Eppendorf, 0030078543)
- 10  $\mu$ L pipette filter tip (Labsolute, 7695880)
- 200  $\mu$ L ART wide bore pipette filter tip (Fisher Scientific, 2069GPK)
- 1000  $\mu$ L 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  $\mu$ L, 100  $\mu$ L, 200  $\mu$ L, 1000  $\mu$ L)
- Timer
- Neubauer counting chamber

**Note:**

- **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**

**A. Reagent and Buffer preparation (for 3 samples)****Collagenase/Dispase**

Prepare in advance. Resuspend the Collagenase/Dispase in nuclease-free water to 100mg/mL. Prepare single-use aliquots (45 µL) and store them at -20°C or -80°C. Once thawed, do not refreeze/reuse.

**Supplemented HBSS**

Prepare in advance, store at 4°C, and warm to 28°C before the start of the experiment.

Ingredient	Final concentration	Amount
HBSS	-	49225 µL
CaCl <sub>2</sub>	0.5 nM	25 µL
HEPES	15 mM	750 µL

**Dilution buffer**

Prepare fresh and keep on ice until use.

Ingredient	Final concentration	Amount
HBSS (unsuppl.)	-	496 µL
BSA	0.04%	4 µL

### Washing buffer

Prepare fresh and keep on ice until use.

Ingredient	Final concentration	Amount
DMEM/F-12	-	3968 $\mu$ L (4x 992 $\mu$ L)
BSA	0.04%	32 $\mu$ L

### Quenching buffer

Prepare fresh and keep at 28°C until use.

Ingredient	Final concentration	Amount
DMEM/F-12	-	1784 $\mu$ L (2x 892 $\mu$ L)
Calf serum	10%	200 $\mu$ L
BSA	0.04%	16 $\mu$ L

### Dissociation buffer

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

Ingredient	Final concentration	Volume
Suppl. HBSS	-	1744 $\mu$ L (2x 872 $\mu$ L)
TrypLE	1X	200 $\mu$ L
Collagenase/Dispase	2 mg/mL	40 $\mu$ L
BSA	0.04%	16 $\mu$ L

## **B. Collection of zebrafish larvae**

Perform the following working steps at room temperature:

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

## **C. Wash zebrafish larvae**

Perform the following working steps under a safety cabinet:

- (1) Carefully take off water without pipetting the larvae and immediately add 1 mL of supplemented and pre-warmed HBSS.
- (2) Carefully take off 900 µL HBSS without pipetting the larvae.
- (3) Add TrypL and Collagenase/Dispase to Dissociation-Mix
- (4) Carefully take off the rest of HBSS without pipetting the larvae using a 300 µL pipette tip

## **D. Dissociate zebrafish larvae**

Perform the following working steps under a safety cabinet:

- (1) Immediately add 500 µL of prewarmed Dissociation buffer.
- (2) Incubate larvae at 28°C for 3 minutes.
- (3) Using a 1 mL tip, pipette up and down the larvae 20 times.
- (4) Incubate larvae at 28°C for 3 minutes.
- (5) Using a 1 mL tip, carefully pipette up and down the larvae 15 times.
- (6) Repeat the pipetting/incubation step every 3 minutes for 5 cycles (15 minutes).
- (7) Add 500 µL Quenching buffer per dissociated ZFE (per tube), and mix by careful pipetting using a normal 1 mL tip 10 times.

## **E. Wash cells**

Perform the following working steps under a safety cabinet:

- (1) Immediately strain the cells carefully through a 70  $\mu$ M FlowMi pipette tip cell strainer into a fresh 1.5 mL LowBind SafeLockTube.
- (2) Spin down cells for 5 minutes at 300 x g and 4°C (acceleration set to 7 and brake to 8) and place on ice.
- (3) Carefully take off the supernatant without disturbing the pellet.
- (4) Add 1 mL of prechilled washing buffer to the cell pellet.
- (5) Carefully resuspend cells by pipetting them up and down using a wide-bore pipette tip and placing them on ice.
- (6) Immediately strain the cells carefully through a 40  $\mu$ M FlowMi pipette tip cell strainer into a fresh 1.5 mL LowBind SafeLockTube.
- (7) Spin down cells for 5 minutes at 300 x g and 4°C (acceleration set to 7 and brake to 8) and place on ice.
- (8) Carefully take off the supernatant without disturbing the pellet.
- (9) Add 100  $\mu$ L of prechilled dilution buffer to the cell pellet (total volume ~ 120  $\mu$ L).
- (10) Carefully resuspend cells by pipetting them up and down using a wide-bore pipette tip and placing them on ice.
- (11) Start cell counting and cell viability estimation.

## **F. Cell viability determination**

Perform the following working steps under a safety cabinet:

- (1) Transfer 10  $\mu$ L Trypan-Blue into a fresh 1.5 mL LowBind SafeLockTube.
- (2) Add 10  $\mu$ L of cell suspension to Trypan-Blue.
- (3) Mix by carefully flicking.
- (4) Apply 10  $\mu$ L of the stained cell suspension to a Neubauer counting chamber
- (5) In four quadrants, count alive and dead cells separately and note the results in the LabTrackingForm.
- (6) Take a picture using the microscope camera for documentation
- (7) Calculate the viability rate.

## **G. Cell counting**

Perform the following working steps under a safety cabinet:

- (1) Apply 10  $\mu\text{L}$  of the unstained cell suspension to a Neubauer counting chamber.
- (2) Count all cells in four quadrants and note the LabTrackingForm results.
- (3) Take a picture using the microscope camera for documentation.
- (4) Calculate the cell number per  $\mu\text{L}$ .

## **H. Quality control**

- (1) Visually check the cell suspension for undissociated tissue and debris. It should be as clean as possible.
- (2) Check if cell concentration is sufficient for the 10X single-cell workflow (> 400 cells per  $\mu\text{L}$ ).
- (3) Check if cell viability is sufficient for the 10X single-cell workflow (> 80%).

## **I. Chip loading**

- (1) Prepare the 10X Chip and MasterMix according to the manufacturer's instructions.
- (2) Strain the cell suspension (~100  $\mu\text{L}$ ) carefully through a 40  $\mu\text{M}$  FlowMi pipette tip cell strainer into a fresh 1.5 mL LowBind SafeLockTube.
- (3) Add cell suspension to MasterMix and start Chip loading.

## **J. Cleaning**

- (1) Clean all used materials and return them to their original place.
- (2) Clean and disinfect the safety cabinet.
- (3) Turn off all used instruments.