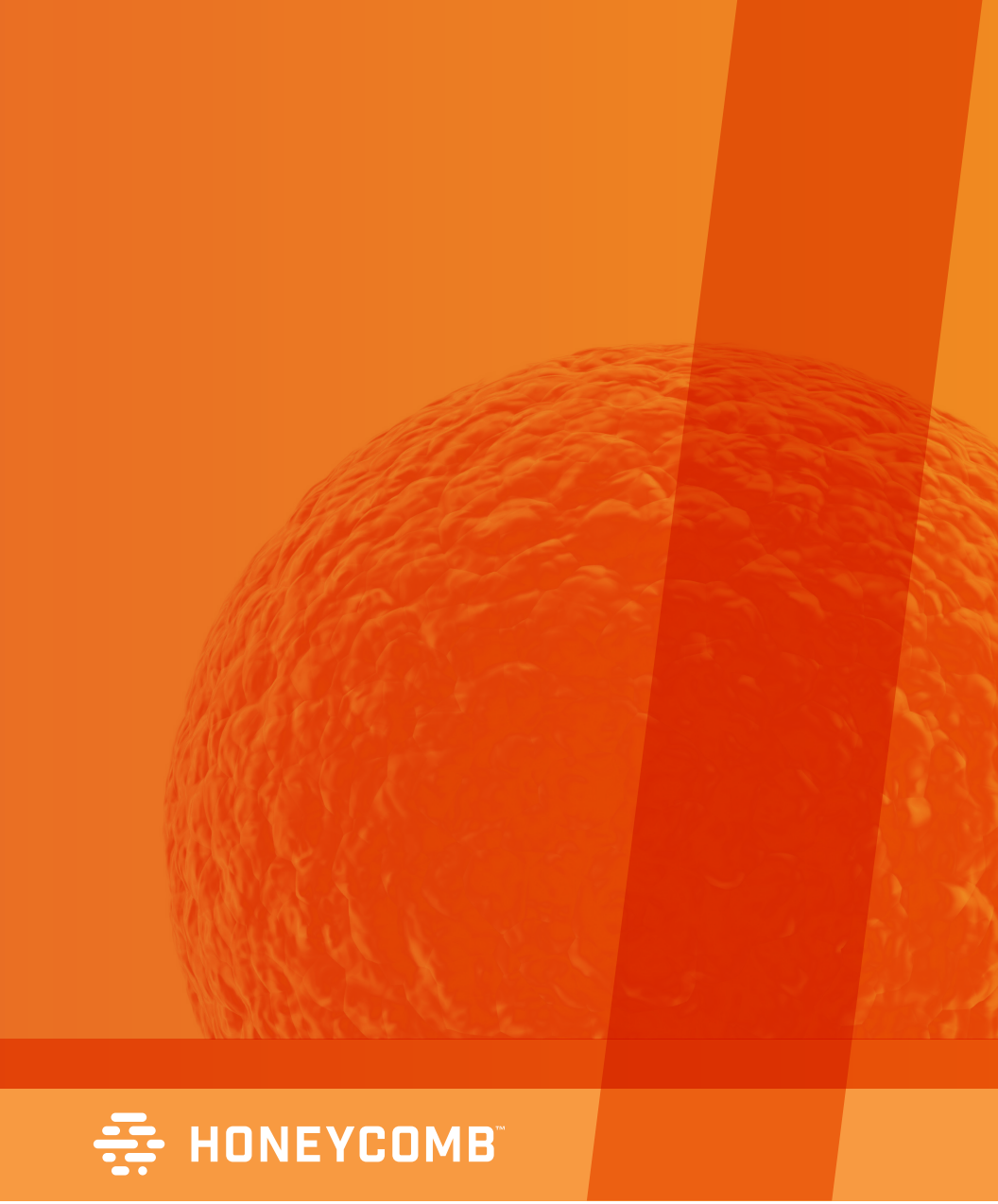


HONEYCOMB™

HIVE™ scRNAseq v1 Sample Capture Training with Cell Surrogates

User Protocol

v21.10

This product is for research use only.
Not for use in diagnostic procedures.

HIVE™ scRNAseq Sample Capture Training with Cell Surrogates:

///// Product Overview

HIVE™ scRNAseq is a complete solution transforming single-cells to NGS libraries. The HIVE is a portable, handheld, single-use device that enables gentle capture, easy storage, and scalable processing for the analysis of single-cell samples. Cell-loaded HIVEs can be stored or shipped until ready for Honeycomb's simplified and scalable HIVE processing and library prep workflow.

The HIVE™ scRNAseq workflow is divided into two parts: sample capture and sample processing to create a sequencing library. The following protocol prepares users for successful execution of the sample capture workflow.

///// Kit Overview

Cell Surrogates are 15 µm colored-beads of known concentrations to be used with the **HIVE™ scRNAseq Sample Capture Kit**. This protocol trains user to **1)** accurately calculate sample concentrations and **2)** prepare samples for loading into the HIVE. There are sufficient materials to train two users.

///// Kit Contents & Storage

Cell Surrogates: 4°C

Name	Amount (2 rxns)
High Concentration Cell Surrogate	250 µL
Low Concentration Cell Surrogate	250 µL

Reagents: Ambient (10-35°C)

Name	Amount
Hemocytometer	8
Sample Wash Solution	20 mL
Cell Preservation Solution	40 mL
Stopper	8

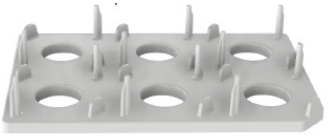
HIVE Collectors: -20°C

Name	Amount
Hive Collector	8

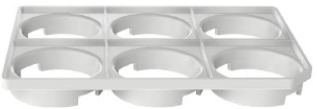
Spin Parts: Ambient

HIVE Blank x4

Spin Plate x2



Spin Lid x2



///// Revision History

Version	Date	Description
v21.10	October 2021	Product Launch

///// User Supplied Materials

Reagents

- Cell Media
- Cell Media + 0.1% FBS

Disposables

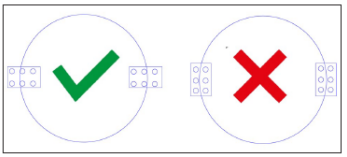
- 1.5 mL microfuge tubes

Pipets & Tips

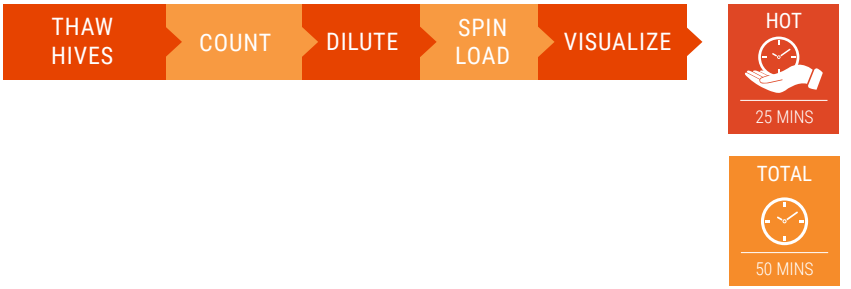
- P1000, P200, P10 Pipets & tips

Equipment

- Light Microscope (inverted preferred to upright), equipped with 4x or 10x objective
- Counting device (or phone app)
- Vacuum Aspirator (optional)
- -20°C freezer
- 4°C refrigerator
- Biosafety cabinet (optional)
- Centrifuge (optional) with plate rotor (or swinging-bucket rotor with plate adaptors), e.g. Eppendorf 5810™ with Rotor S-4-104 and MTP/Flex buckets
    - Critical requirements:
        - 30-1,800 RCF capacity
        - Deep-well plate (DWP) compatible
        - Radial (not perpendicular) plate orientation (see Diagram below)



///// Workflow



10 MINS | TOTAL 30 MINS

STEP A: Calculating Cell Surrogate Concentrations

! **IMPORTANT:** Cell Surrogates settle quickly, mix by pipetting prior to any transfer
! **IMPORTANT:** Range for accurate counting with the hemocytometer = 200-2,000 cells/µL

Prepare HIVE Collectors

1. Remove 2 HIVE Collectors per user from -20°C and from packaging
2. Thaw for 30 minutes at room temperature

During Thaw Complete STEP A

Make 1:10 Dilution of High Concentration Cell Surrogate

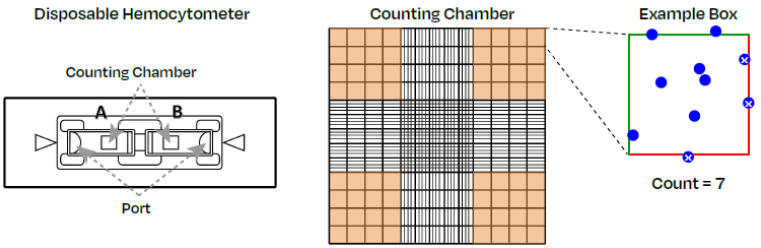
3. Label 1.5 mL tube "1:10 High"
4. Add 90 µL Cell Media + 0.1% FBS to "1:10 High" tube
5. Mix High Concentration Cell Surrogate by pipetting and add 10 µL to "1:10 High" tube

Load Hemocytometer

6. Mix "1:10 High" by pipetting and add 10 µL into **Port A** of the disposable hemocytometer
7. Mix Low Concentration Cell Surrogates by pipetting and add 10 µL into **Port B**, no dilution needed

Count & Determine Concentration

8. Count Cell Surrogates in **four quadrants** of hemocytometer (orange), **16 boxes** in each quadrant
   Do Count all Cell Surrogates fully inside each box, touching the top, and/or left sides of each box.
   Do Not Count any Cell Surrogates touching the bottom and/or right sides of each box



Sample Name	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4	Average Count
"1:10 High"					
"Low"					

9. Calculate Cell Surrogate Concentration (cells/µL)

Cell Surrogate per µl = Average Count x Volume Factor (always 10) x Dilution Factor (ex. 10 for 1:10)

Sample	Expected (cells/µL)	Calculated (cells/µL)
High Concentration Cell Surrogate	2,000/µL	
Low Concentration Cell Surrogate	2,00/µL	

STEP B: Cell Surrogate Preparation & HIVE Loading

5 MINS | TOTAL 10 MINS

! **IMPORTANT:** Cell Surrogates settle quickly, mix well by pipetting prior to any transfer

! **IMPORTANT:** Keep HIVEs flat at all times, only tilt when directed to do so

Prepare Cell Surrogates for Loading (15,000 per HIVE in 1 mL)

1. Label 1.5 mL tubes "15k High" and "15k Low", add 1 mL Cell Media + 0.1% FBS to each tube
2. Calculate volume of the High and Low Concentration Cell Surrogate samples needed for 15,000

$$\frac{(\#\text{ of HIVEs} \times 15{,}000)}{(\text{Cells}/\mu\text{L})}$$

Example $\frac{(1 \times 15{,}000)}{(200/\mu\text{L})} = 75\ \mu\text{L}$

Sample	Concentration (cells/µL) from page 6	Sample Volume (µL)
High Concentration Cell Surrogate		
Low Concentration Cell Surrogate		

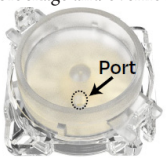
3. Add calculated volumes of each cell surrogate sample to the corresponding tube of 1mL cell media

Load HIVE

4. Label HIVE Collector with sample names "15k High" and "15K Low" on white sticker
5. Remove (and set aside) Stopper from HIVE Collector port 

STEPS 6-8 One HIVE at a Time

6. Tilt the HIVE Collector towards you to remove thawed liquid (~1 mL) through port by pipette or aspiration. Follow the angle of the port with your pipet tip until gently touching the edge of the array leaving space between the pipet tip and the port for air to flow in and out
7. With the HIVE Collector flat, mix "15k High" prepared sample by pipetting, then add through port. If cell media bubbles up, remove and pat port dry with paper towel, try again with fresh pipet tip
8. Fill up the HIVE Collector by adding about 3mL Cell Media (no FBS added). Make sure to tilt the HIVE Collector away from you while adding additional cell media to prevent blockage and overflowing at the port.



9. Repeat STEPS 6-8 with second HIVE and "15k Low" prepared sample
10. Re-insert Stopper into all HIVE Collector port. Some liquid may overflow, pipet the excess solution to remove
11. Place HIVE Collectors on the Spin Plate, open corners to plate pins, balance with HIVE Blank(s) if needed
12. Place Spin Plates with HIVE Collectors in centrifuge, balance with additional Spin Plate + HIVE Blanks if needed
13. Spin at 30 RCF for 3 minutes

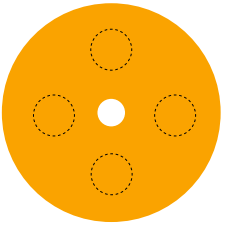
STEP C: Determine Loading Efficiency (optional)

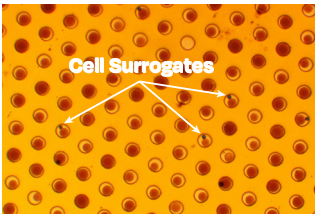
10 MINS | TOTAL 10 MINS

! **IMPORTANT:** Keep HIVEs flat at all times
! **IMPORTANT:** See Appendix if working with an upright rather than inverted microscope
! **IMPORTANT:** Save used HIVE Collectors for practice with HIVE parts in Processing Kit

1. Using a 4x or 10x objective count **all** Cell Surrogates visible in 4 fields of view across the array

HIVE Array



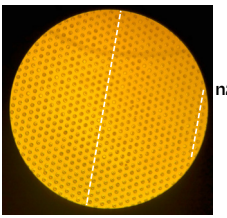


Example field of view locations

Sample	Field 1	Field 2	Field 3	Field 4	Average Count
"15k High"					
"15k Low"					

2. Estimate the number of wells per field (only need to do for one field of view)
   a. count the number wells at the widest point in the field (**n1**)
   b. count the number wells at the narrowest point in the field (**n2**)

n1 (*the largest number of wells*)



n2 (*the smallest number of wells*)

# of wells per field = n1(n1-1) - (n2-1)(n2-2) **Example** 28(28-1) - (10-1)(10-2) = *684 wells*

$\left(\frac{\text{Average Cell Count}}{\text{\# of wells per field}}\right) \div 0.23 = \text{loading efficiency}$ **Example** $\left(\frac{134}{684}\right) \div 0.23 = 0.85$

3. Calculate Cell Surrogate loading efficiency, ideally between 0.50-1.50

STEP A
STEP B
STEP C

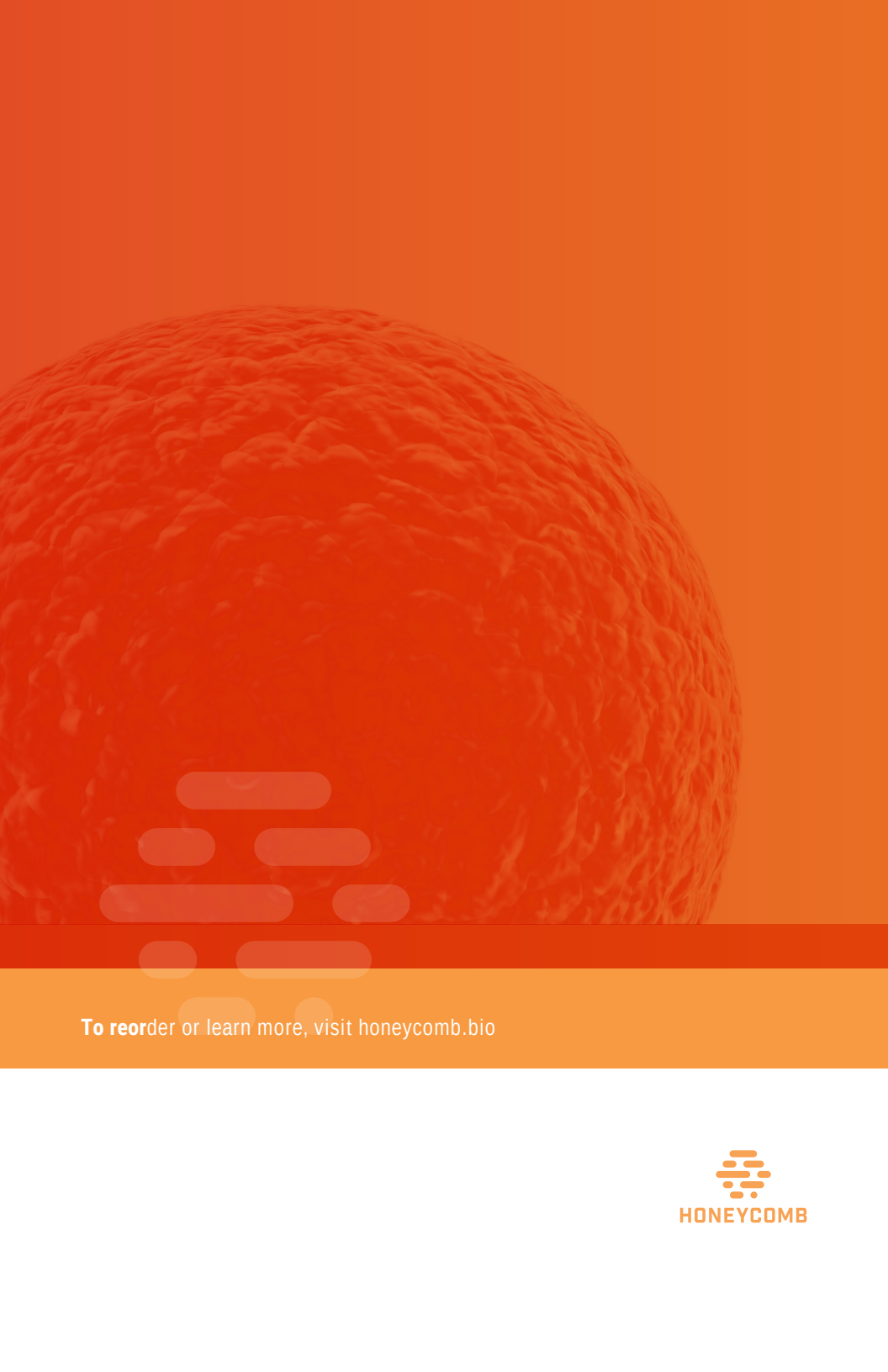
Cell-loading by gravity (without a centrifuge)

1. Once all HIVE Collectors are loaded, incubate for 15 minutes at 4°C (in fridge), to allow cell surrogates to settle

Working with an upright microscope

1. Removal of the Cell Loader is required for visualization of the array
2. Push out on HIVE base tabs while lifting up on Cell Loader wings

3. If working with gravity (not centrifugation) cell-loaded HIVEs, add 4 mL of PBS to the trough around the array to prevent it from drying out during imaging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