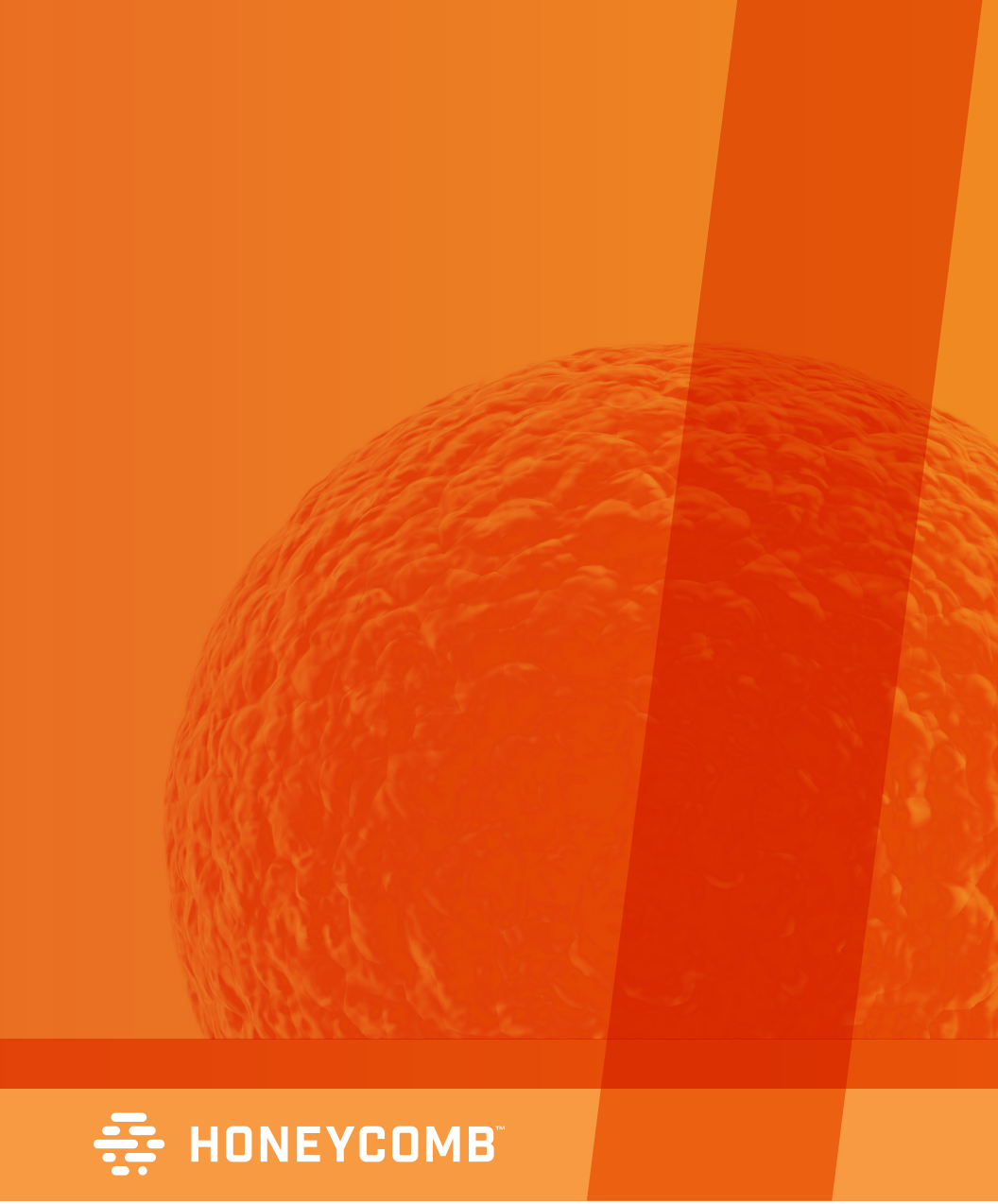


HONEYCOMB™

HIVE™ scRNAseq v1 Sample Capture Kit

User Protocol - Revision A

v22.09 | September 2022 | Revision A

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

HIVE™ scRNAseq Sample Capture Kit:

///// 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 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 guides users through sample capture and storage and/or shipping of cell loaded HIVEs.

///// Kit Overview

The **HIVE™ scRNAseq Sample Capture Kit** is provided with enough parts and reagents for 8 samples. HIVE Collectors have a picowell array of more than 65,000 wells , 60 µm in diameter, pre-loaded with barcoded 3' transcript capture beads.

///// Kit Contents & Storage

Reagents: Ambient (10-35°C)

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

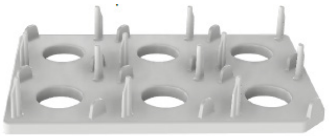
HIVE Collectors: -20°C

Name	Amount
Hive Collector	8

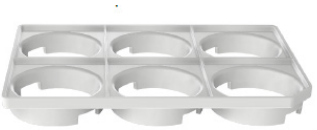
Spin Parts: Ambient

HIVE Blank x4

Spin Plate x2



Spin Lid x2



///// Revision History

Version	Date	Description
v21.10	October 2021	Product Launch
v22.09 | Revision A	September 2022	Addition of shipping & receiving information

///// User Supplied Materials

Sample Preparation

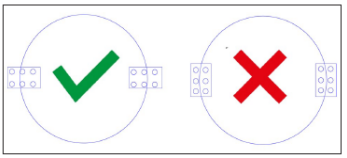
- User defined reagents, disposables, and equipment

Pipets & Tips

- P1000 Pipets & tips

Equipment

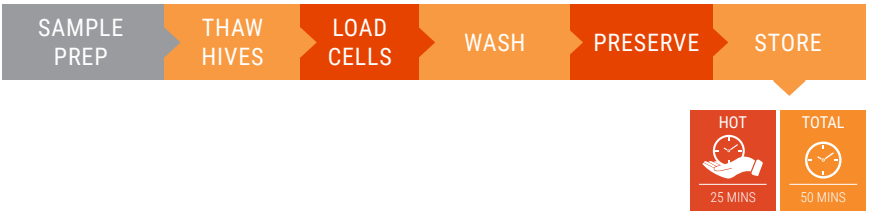
- -20°C freezer
- 4°C refrigerator (optional)
- Biosafety cabinet (optional)
- Centrifuge (optional) with plate rotor (or swinging-bucket rotor with plate adaptors), e.g. Eppendorf 5810™ with Rotor S-4-104 and MTP/Flex bucketss
  - Critical requirements:
    - 30-1,800 RCF capacity
    - Deep-well plate (DWP) compatible
    - Radial (not perpendicular) plate orientation (see Diagram below)



Optional

- Vacuum Aspirator
- Nunc™ Square BioAssay Dishes. Thermo Scientific (CAT# 240845)

///// Workflow



10 MINS | TOTAL 30 MINS

STEP A: HIVE & Sample Preparation

IMPORTANT: Cells will settle quickly, mix immediately prior to any transfer
IMPORTANT: Poor cell viability leads to poor quality data, recommend >90% viability
IMPORTANT: Recommended loading 15,000 cells in 1 mL per HIVE

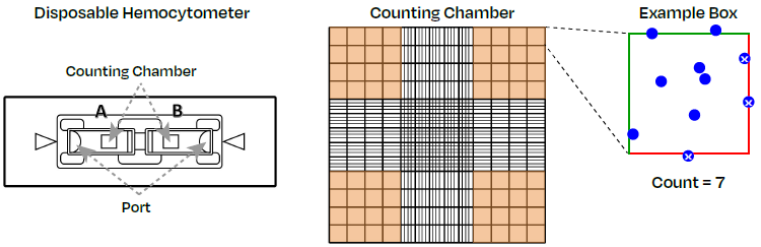
Prepare HIVE Collectors

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

Complete Sample Preparation During Thaw

Preparation of Sample(s) for Loading

3. Follow user defined protocols for generation of single-cell suspension in desired cell media
4. Disposable hemocytometers are provided for calculating sample concentration
5. Load 10 µL of single-cell suspension into hemocytometer port
   Dilute some of your sample if needed for accurate counting: 200-2,000 cells/µL
6. Count cells in **four quadrants** of hemocytometer (orange), **16 boxes** in each quadrant
   Do Count all cells fully inside each box, touching the top, and/or left sides of each box
   Do Not Count any cells touching the bottom and/or right sides of each box



7. Calculate average cell count

Sample Name	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4	Average Count

8. Calculate sample concentration (cells/µL)
   Cells per µL = Average Count x Volume Factor (always 10) x Dilution Factor (ex. 10 for 1:10)

9. Calculate cell media and sample volume for loading HIVE

Cell Media Volume

# of HIVEs x 1ml **Example** 10 x 1ml = ***10ml***

Sample Volume

$$\frac{(\#\text{ of HIVEs} \times 15{,}000\text{ cells})}{(\text{Cells}/\mu\text{L})}$$ **Example** $$\frac{(10 \times 15{,}000)}{(1{,}000/\mu\text{L})} = 150\mu\text{L}$$

Sample Name	Concentration (cells/µL)	# of HIVEs	Cell Media Volume (mL)	Sample Volume (µL)

STEP A
STEP B
STEP C

STEP B: Cell Loading in the HIVE

10 MINS | TOTAL 15 MINS

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

IMPORTANT: A vacuum aspirator is recommended for liquid removal

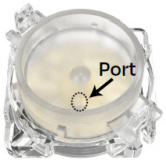
Load HIVE

1. Label HIVE Collector with your sample name on the white sticker and record HIVE serial number
2. Remove (and set aside) Stopper from HIVE Collector port

STEP A

STEPS 3-4 One HIVE at a Time

3. 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.



4. With the HIVE Collector flat, mix sample by pipetting, then add 1mL through port. If cell media bubbles up, remove and pat port dry with paper towel, try again with fresh pipet tip
5. 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.
6. Re-insert Stopper into all HIVE Collector port. Some liquid may overflow, pipet the excess solution to remove.
7. Put HIVE Collectors on the Spin Plate, open corners to plate pins, balance with HIVE Blank(s) if needed
8. Place Spin Plates with HIVE Collectors in centrifuge, balance with additional Spin Plate + HIVE Blanks if needed
9. Spin at 30 RCF for 3 minutes

STEP B

Washing

STEPS 10-12 One HIVE at a Time

10. Remove (and discard) Stopper from HIVE Collector port
11. Tilt the HIVE Collector towards you and remove 2 mL of the cell media. Gently shake the HIVE Collector to pop any large bubbles before removing the rest of the cell media
12. With the HIVE Collector flat, add 2 mL Sample Wash Solution

Molecular Preservation

STEP C

STEPS 13-15 One HIVE at a Time

13. Tilt the HIVE Collector towards you to remove Sample Wash Solution
14. With the HIVE Collector flat, add 1mL Cell Preservation Solution
15. Insert a new Stopper into HIVE port

STEP C: Cell-loaded HIVE Storage, Shipping, & Receiving

5 MINS | TOTAL 35 MINS

! **IMPORTANT:** Keep HIVEs flat at all times until fully frozen (not fully frozen at -20°C)
! **IMPORTANT:** 3-5 Kg of dry-ice per day are recommended for shipping

Storage

Place cell-loaded HIVEs back in original packaging and store at -20°C until ready for processing with **HIVE™ scRNAseq Processing Kit.**

Note: *If continuing immediately to the processing protocol, incubate in the cell preservation solution for 30 minutes at room temperature instead of freezing.*

Shipping

1. Cover HIVEs in box with absorbent sheets, tape box closed, place in ziplock bag, and freeze
   a) at -80°C for 30 minutes to fully freeze while flat prior to packing in styrofoam box with dry-ice
   OR
   b) in styrofoam box, covered with dry-ice for 30 minutes to fully freeze while flat before moving
2. It is recommended to photograph the frozen HIVEs before shipping for QC
3. Ship cell-loaded HIVEs according to **IATA instructions**, consult your institute's guidelines
   - Shipping of category B substances need to be done in triple waterproof packaging
   - Absorbent material is required for Biological Substance, Category B (UN 3373) shipments
   - Place absorbent material between the primary and secondary receptacles, using enough material to absorb the entire contents of all primary receptacles
4. Place styrofoam box (do not seal or tape closed) inside of a cardboard box for acceptance by carrier
5. If reusing a box, remove all markings and labels
6. Determine weight of dry-ice
7. Create packing list of contents with descriptions, volumes, and quantities, for example:

Item	Name	Quantity
1	Watertight sample container, with healthy human donor leukocytes from blood, filled with cell preservation solution categorized as non dangerous goods. Total volume of 1ml per container.	8

8. Recommended labeling on outer packaging
   a) Biological substance Category B UN3373
   b) Dry-ice label with weight
   c) Red up arrows
   d) Fragile
9. Packages should be shipped overnight on a Monday to avoid weekend delays
10. It is recommended the use of a courier service to ensure dry-ice replenishment and correct documentation, especially for international shipping

Receiving

On dry ice, cell-loaded HIVEs should be checked to make sure:

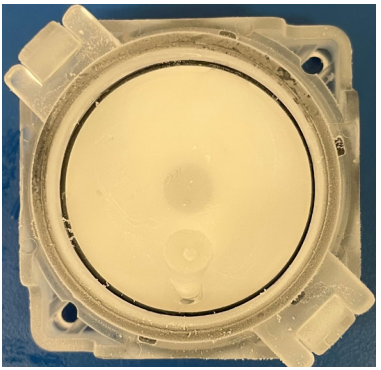
1. Dry ice remains in the package, any temperature sensors have not been activated, and the HIVE is still frozen
2. The liquid in the HIVE is completely covering the HIVE array and is white in color
3. The clear stopper is still present

IMPORTANT! Cell-loaded HIVEs should be placed at **-80** immediately to ensure they remain frozen

It is recommended to photograph the frozen HIVEs before and after shipping for QC.

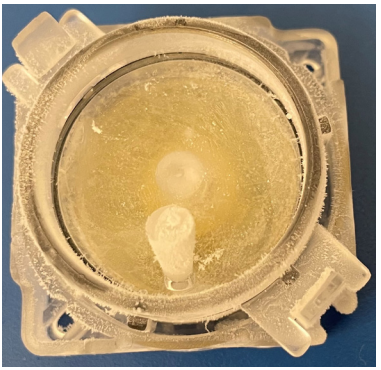
Example Photos:

STEP A



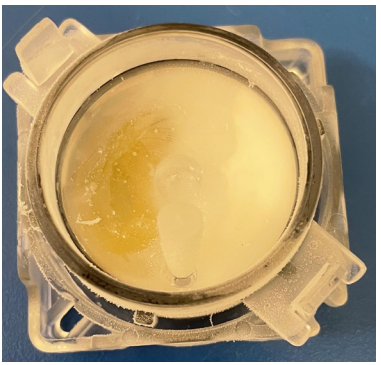
Good (liquid is frozen, white in color, and completely covers the array)

STEP B



Bad (frozen liquid is clear- cell preservation solution was not used)

STEP C



Bad (frozen liquid does not completely cover the surface and the yellow array is visible)

10 MINS | TOTAL 55 MINS

Alternative STEP B: Cell-loading by gravity (without a centrifuge)

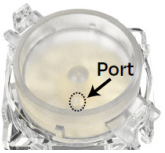
! **IMPORTANT:** Keep HIVEs flat at all times, only tilt when directed to do so

Load HIVE

1. Label HIVE Collector with your sample name on the white sticker and record HIVE serial number
2. Remove (and set aside) Stopper from HIVE Collector port 

STEPS 3-4 One HIVE at a Time

3. Tilt the HIVE Collector, 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



4. With the HIVE Collector flat, mix sample by pipetting, then add 1mL through port. If cell media bubbles up, remove and pat port dry with paper towel, try again with fresh pipet tip
5. Once all HIVE Collectors are loaded, incubate for 30 minutes at 4°C (in fridge)

Washing

STEPS 6-7 One HIVE at a Time

6. Tilt the HIVE Collector and remove cell media
7. With the HIVE Collector flat, add 1 mL Sample Wash Solution

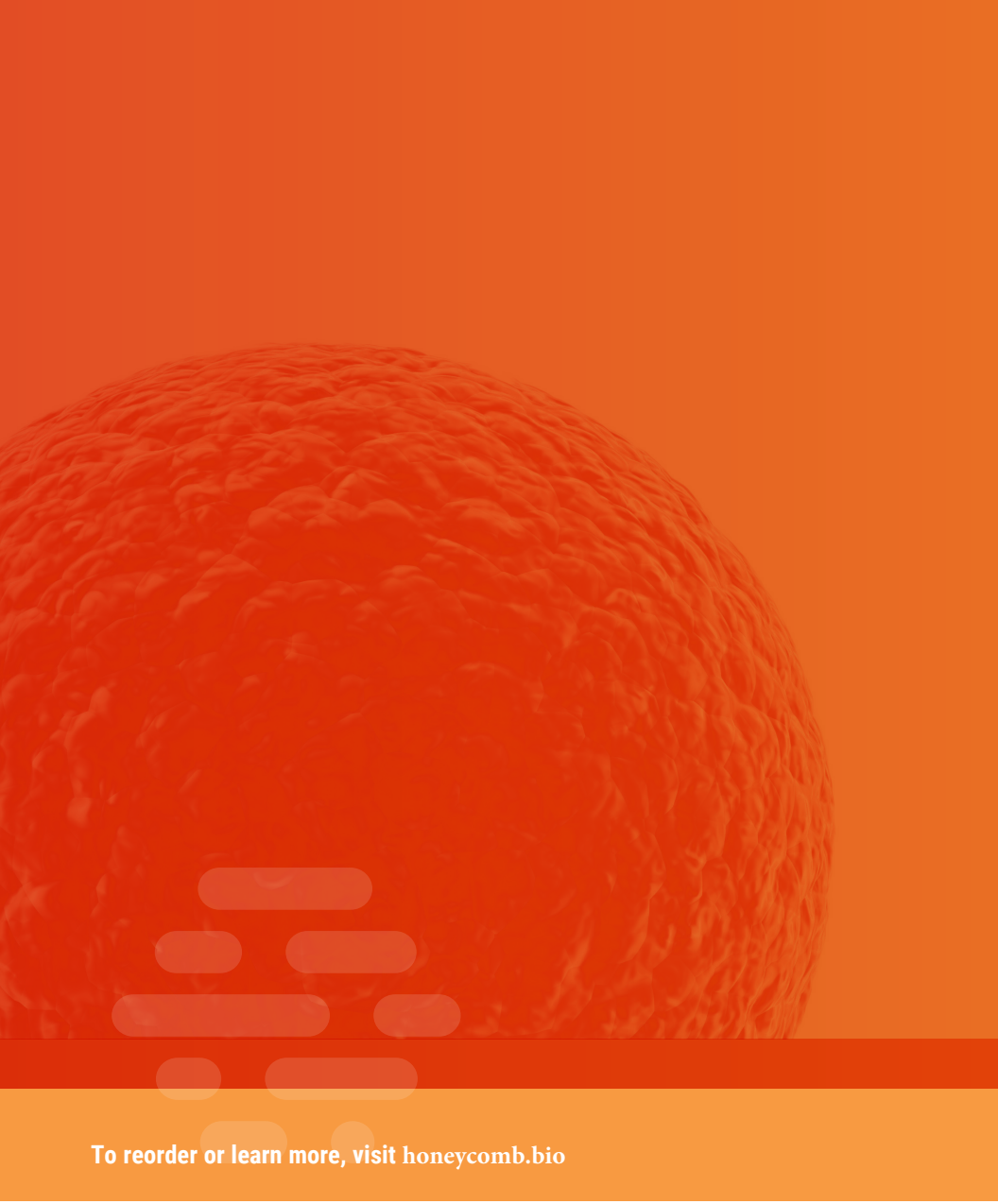
STEPS 8-10 One HIVE at a Time

8. Tilt the HIVE Collector and remove Sample Wash Solution
9. With the HIVE Collector flat, add 1mL Sample Wash Solution again
10. Once all HIVE Collectors are filled, incubate for 15 minutes at room temperature

Molecular Preservation

STEPS 11-13 One HIVE at a Time

11. Tilt the HIVE Collector and remove Sample Wash Solution
12. With the HIVE Collector flat, add 1mL Cell Preservation Solution
13. Insert new Stopper into HIVE port



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