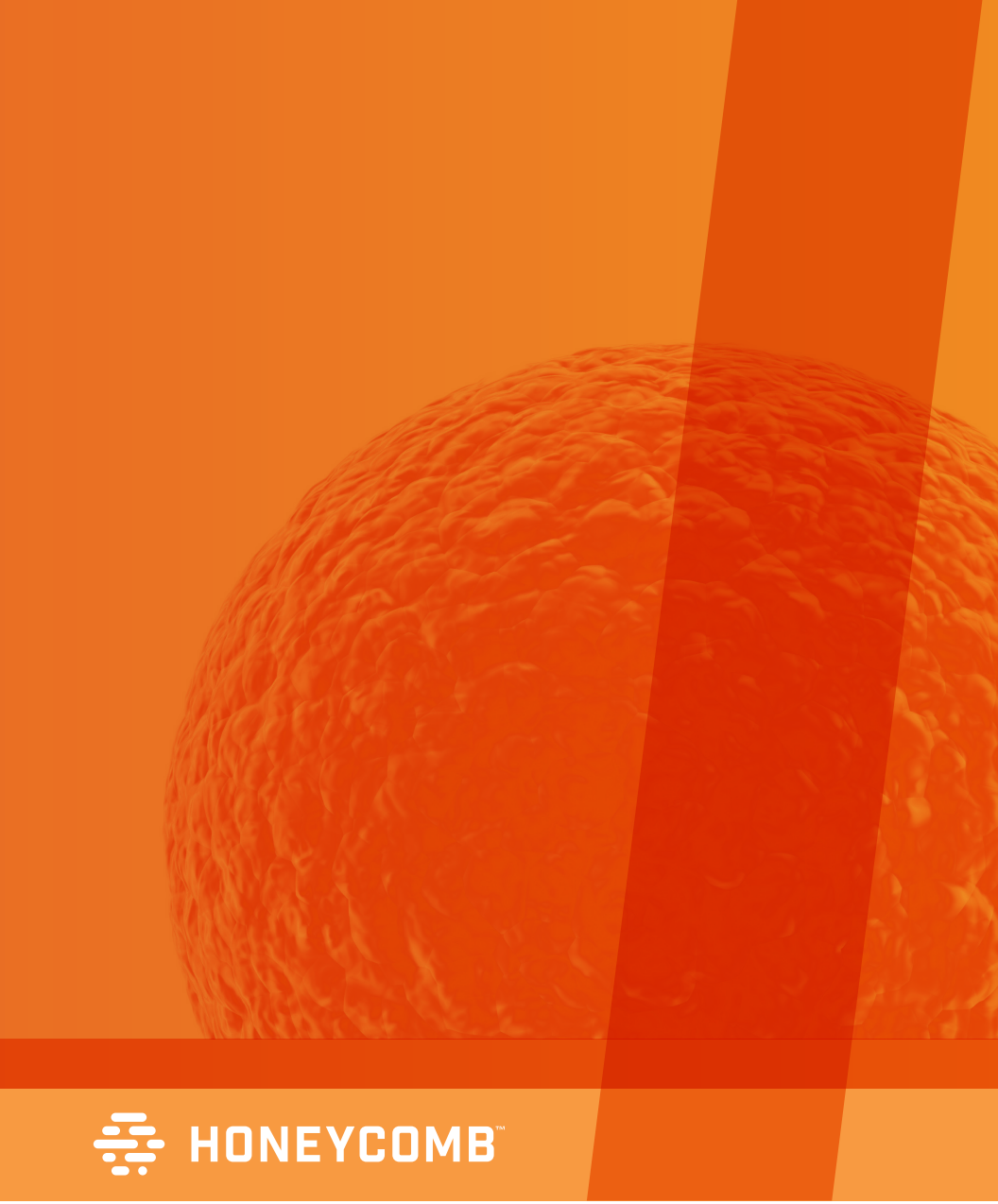


HONEYCOMB™

Getting Started with HIVE™ scRNAseq v1

v21.10

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

Getting Started with HIVE™ scRNAseq

///// 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.

///// HIVE Features & Potential Applications

Integrated sample storage: Store cell-loaded HIVEs after Sample Capture at -20°C until ready for processing and library preparation. Ship on dry-ice (For up-to-date storage duration, please see FAQs at honeycomb.bio).

Large sample loading volume: Ideal for sparse samples such as FACS enrichment, FNAs, other peripheral biopsies (1-4 ml recommended loading volume range).

Fragile cell recovery: Gentle sample capture, and robust sample storage enable recovery of fragile cells (such as neutrophils, eosinophils, and basophils).

Flexible and scalable sample number: Process 1 to 24 samples, in parallel, in ~12 hrs.

Strong lysis solution: High concentration reducing agent for denaturing proteins and working with infectious disease samples.

///// Kit Overview

The **HIVE™ scRNAseq Starter Bundle** includes consumables and accessories for first time users. When combined with the HIVE™ scNRAseq Sample Capture and Processing Kits, there are enough HIVE parts and reagents for 8 samples.

Using the Cell Surrogates and Molecular Controls, there are enough HIVE parts and reagents to:
A. train 1 user, and run 4 samples
B. train 2 users, and run 2 samples

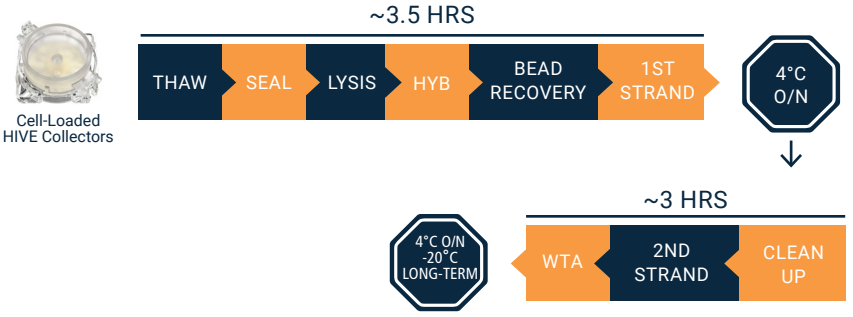
Product List	Contents	Description
Sample Capture Kit	HIVE Collectors, Reagents, Spin Parts	Parts and reagents for sample capture
Cell Surrogates	High and low concentration cell surrogates	Sample capture training materials
Processing Kit	HIVE Parts & Reagents, Library Prep Reagents, Spin Parts	Parts and reagents for processing/lib prep
Molecular Controls	Pre- and Post-1st Strand, WTA Input, and Index PCR Controls	Processing/library prep training materials
HIVE Accessories	Closure tool, filter plate adaptor	Custom HIVE accessories
Index Plate	96 unique index pairs	For multiplexing HIVE libraries
Vacuum Kit	Vactrap and manifold	Pre-assembled vacuum set-up
Plate Kit	Filter, deep-well , and full-height plates, foil & clear seals	Commercially available plates and seals
Lysis Boxes	Reusable plastic boxes	For odor prevention during lysis
BeeNet™	Custom analysis software to generate count matrices	Required for HIVE scRNAseq libraries

///// Revision History

Version	Date	Description
v21.10	October 2021	Product Launch

///// Sample Capture

///// Processing Day 1

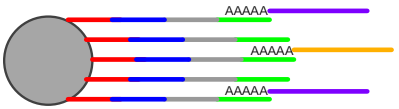


///// Processing Day 2

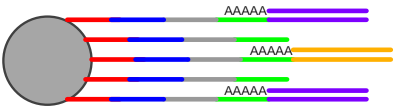
///// ANALYSIS with BeeNet™ Software

Bead Oligos
Universal Primer Sequence (UPS)
Cell Barcode
Random Linker Sequence
Transcript Capture Sequence - poly(dT)

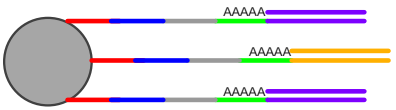
1. Hybridization: Capture poly-A transcripts



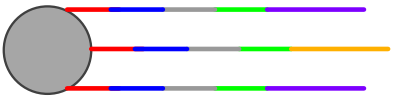
2. 1st Strand Synthesis: Bead oligos acts as primer for making 1st-strand cDNA



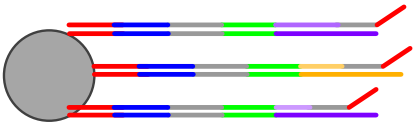
3. Bead Clean-Up: Remove any bead oligos without 1st strand cDNA



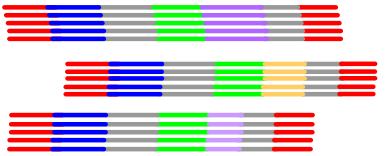
4. NaOH denaturation: Makes 1st strand cDNA single-stranded



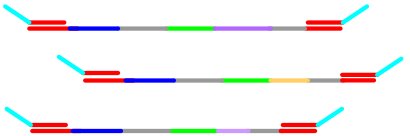
5. 2nd Strand Synthesis: Randomly prime synthesis of 2nd strand cDNA



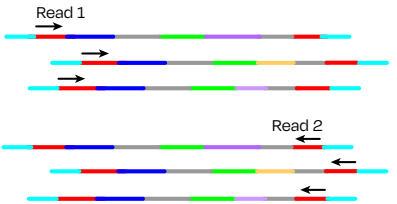
6. WTA: Amplify 2nd strand cDNA with UPS primers



7. Index PCR: Add P5+ i5 and P7+i7 to WTA product with UPS primers , for library multiplexing and Illumina® sequencing



8. Sequencing: Read 1 for cell barcode, and Read 2 for transcript identity



Reagents

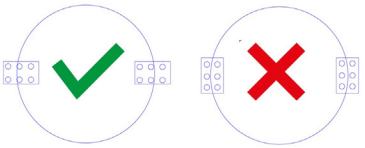
- Molecular biology grade ethanol, absolute.
- Wescodyne® (bleach alternative).

Disposables

- Reagent reservoirs for 25-50 mL
- Paper towels
- Optional: Nunc™ Square BioAssay Dishes. Thermo Scientific (CAT# 240845)

Equipment

Radial (Not Perpendicular) Plate Orientation



- -20°C freezer
- 4°C refrigerator and ice bucket
- Biosafety cabinet (optional)
- Oven, for 37°C and 50°C incubations
- Bench-top vortex
- Centrifuge 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:
  - 1,800 RCF capacity
  - Deep-well plate (DWP) compatible
  - Radial (not perpendicular) plate orientation (see Diagram above)
- Thermocycler for 96-well plate
- Bar magnet for 96 well plates, e.g. Invitrogen DynaMag™-96 Side Skirted (CAT# 12027)
- DNA quantification device, e.g. Thermo Scientific QuBit™ 4 Fluorometer (CAT# Q33238)
- DNA capillary electrophoresis device, e.g. Tapestation™, Bioanalyzer,™ or LabChip GX Touch™ (plus kit for >1,000bp DNA smear)

Pipets & Tips

- Pipet aid (optional) - 5 mL - 25 mL serological pipettes
- Single-channel 1000 µL - 1000 µL tips
- 8-channel and single-channel 200 µL, single-channel 20 µL - 200 µL tips
- 8-channel and single-channel 10 µL - 10 µL tips

Currently Available from Revvity

Plate Kit (sufficient for 2 experiments)

- 96-well filter plates. Millipore MultiScreen™ Filter Plates (CAT# MSHVN4B10)
- 96-well deepwell plate. Fisher Sci round well, V/U/conical bottom, >0.8mL well capacity, natural polypropylene (CAT# AB0765)
- 96-well full-height PCR plate, 0.3ml metric capacity. Thermo Fisher semi-skirted, flat deck, black lettering (CAT# AB1400L)
- Evaporation resistant adhesive PCR plate sealing films. Biorad Microseal™ 'B' adhesive film (CAT# MSB1001)
- Adhesive foil PCR plate seal. Excel Scientific, eXTReme™ FoilSeal™ (CAT# XTR-FOIL-100)

Vacuum Kit

- Vacuum pump/line* (optional), e.g. Cole-Parmer Air diaphragm vacuum/pressure pump, 0.37 cfm, 115 VAC (CAT# EW-79202-00)
- Vacuum aspiration reservoirs, e.g. VWR Vactrap™ Vacuum Trap System for Aspiration and Vacuum Protection (CAT# 76207-602)
- 96 well vacuum manifold, e.g. Millipore MultiScreen™ Vacuum Manifold 96-well (CAT# MAVM0960R)

*can reach vacuum of at least 15 in Hg (381 mm Hg), and fit tubing with inner diameter of 0.25 inches (0.63 cm)

To reorder or learn more, visit honeycomb.bio

HONEYCOMB