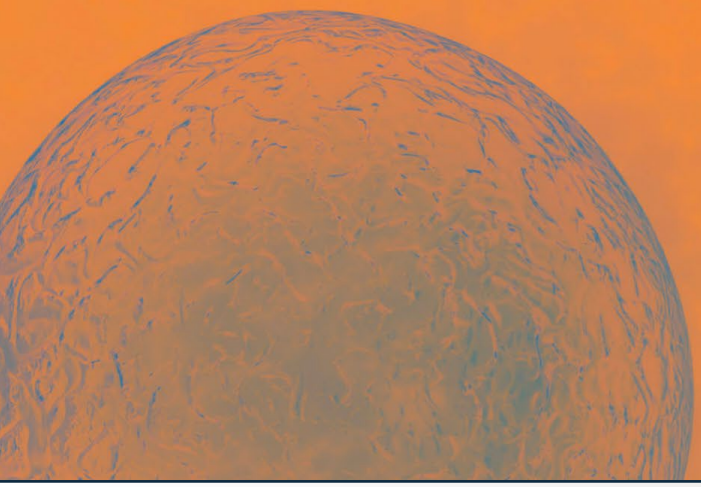

HONEYCOMB

HIVE CLX™ scRNAseq Sample Capture

User Protocol

Version 1.0 | May 2023

Any cell. Any where. Any time.™

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

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Revision History

Version	Date	Description
Version 1.0	May 2023	CLX Product Launch

HIVE CLX™ scRNAseq Sample Capture

GENERAL INFORMATION

PRODUCT OVERVIEW

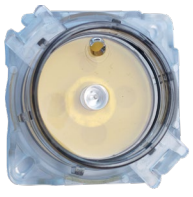
HIVE CLX scRNAseq is a complete solution to create NGS libraries from single cells. This Sample Capture Kit includes handheld, single-use HIVE Collectors for gentle cell capture and scalable sample processing. Each HIVE contains a picowell array that is pre-loaded with barcoded capture beads for 3’-transcripts.

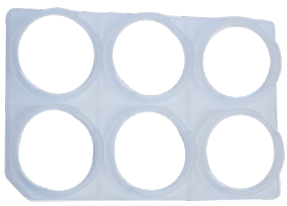
The HIVE CLX scRNAseq Sample Capture product contains enough parts and reagents for 8 samples. Loaded HIVE Collectors can be stably stored and shipped for seamless library prep and sequencing.

KIT CONTENTS & STORAGE

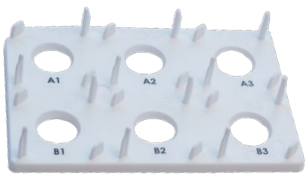
//// HIVE Collectors stored at -20°C (×8)
\\\\ Blue Stoppers (×8)
//// CLX Sample Wash Solution (50 mL)
\\\\ CLX Cell Preservation Solution (20 mL)
//// Disposable Hemocytometers (×8)
\\\\ HIVE Spin Plates (×2)
//// HIVE Spin Lids (×2)
\\\\ HIVE Blanks (×4)

IMPORTANT!
Store HIVE Collectors in -20°C or -80°C freezer. Keep all other contents at ambient temperature (10–35°C).


HIVE Collector


HIVE Spin Lid


HIVE Blank


HIVE Spin Plate

Hemocytometer

NOTE

We recommend using PBS + 0.1% BSA as loading media for maximum cell recovery. Use an alternative cell media if your specific sample type is incompatible with PBS + 0.1% BSA.

USER-SUPPLIED MATERIALS

//// Cell Media (PBS recommended)
\\\\ Cell Media + 1% FBS or 0.1% BSA
(PBS + 0.1% BSA recommended)
//// P1000 (1 mL) pipette & tips
\\\\ P20 (20 µL) pipette & tips
//// Absorbent paper towels
\\\\ Shipping materials (optional): Large resealable plastic bag, dry ice (30 lb for 3 days in shipping), Styrofoam box, cardboard box, tape, labels

NOTE

Page 10 offers protocols for loading samples with a centrifuge or by gravity in a refrigerator.

REQUIRED EQUIPMENT

//// -20°C freezer
\\\\ Centrifuge or 4°C refrigerator
//// Brightfield microscope to count cells

NOTE

Follow your institution's biosafety protocols for sample handling, which may include using a biosafety cabinet.

OPTIONAL EQUIPMENT

//// -80°C freezer or dry ice for shipping
\\\\ Biosafety cabinet for sample handling
//// Nunc™ Square Bioassay Dishes for HIVE handling
\\\\ Vacuum aspirator

CENTRIFUGE SPECIFICATIONS

//// 30 RCF spin speed
\\\\ Swinging-bucket centrifuge
//// Compatible with deep-well plates
\\\\ Radial plate orientation (see diagram below)

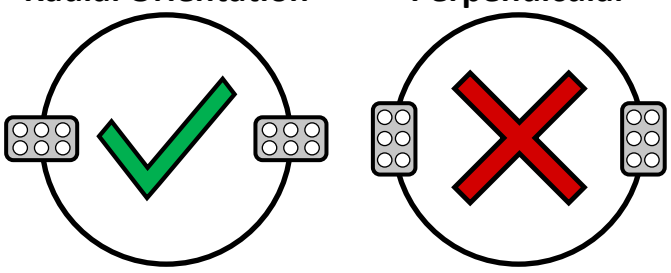
NOTE

Example of compatible centrifuge: Eppendorf 5810™ with S-4-104 rotor and MTP/Flex buckets.

NOTE

If laboratory equipment is limited to the perpendicular orientation, then use positions A2 and B2 only on the HIVE Spin Plate.

Plates must be loaded into the centrifuge in a radial orientation



SAMPLE PREPARATION

SAMPLE REQUIREMENTS & RECOMMENDATIONS

IMPORTANT!
≥90% cell viability gives best data.
Poor viability causes poor data quality.

//// Sample must be a single cell suspension in media
\\\\ Media must contain at least 1% FBS or 0.1% BSA
//// See Appendix 2 for loading recommendations

MEASURE CELL CONCENTRATIONS

NOTE
We recommend washing the cells once with cell media (supplemented with protein) prior to loading.

1. Follow user-defined protocols to create single cell suspensions.
2. Open disposable hemocytometer package and place one hemocytometer onto a flat surface.

IMPORTANT!
Cells will settle quickly.
Mix the sample immediately before any transfer.

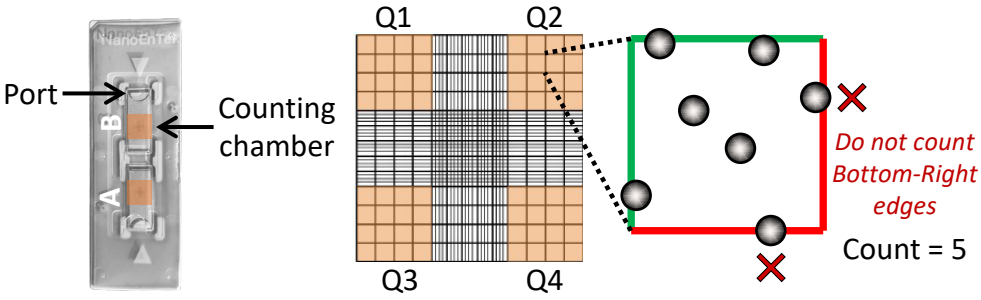
3. Mix sample with gentle pipetting, then pipet 10 µL into Chamber A of the disposable hemocytometer.
4. Mount the hemocytometer to a microscope, then observe each quadrant of the counting chamber.
   - Count all 4 quadrants (16 boxes/quadrant).
   - Count cells fully inside a box or that touch the top or left sides of a box. Do not count cells touching the bottom or right sides.
   - For accurate counting, you may need to dilute a portion of the sample to make 20–200 cells per quadrant, then repeat Steps 2–4 using Chamber B.

NOTE
20–200 cells/quadrant
= 200–2,000 cells/µL

5. Complete tables on next page; then use results to dilute samples with cell media containing protein.
   - We recommend using PBS + 0.1% BSA for dilutions.

NOTE
Diluting 50 µL sample to 500 µL is a 10× dilution factor. Record dilution factors on next page.

Hemocytometer **Counting Chamber** **Example Box**



CALCULATE SAMPLE CELL CONCENTRATIONS

Sample ID	Counts in Hemocytometer Quadrant				Average Cell Count (cells)	Dilution Factor	Sample Concentration (cells/µL)
	Q1	Q2	Q3	Q4			
Example	*3*	*5*	*5*	*7*	*5*	*10*	*500*

Dilution Factor *= Diluted Volume / Sample Volume*

Sample Concentration (cells/µL) *= Average Cell Count × Dilution Factor × 10*

CALCULATE DILUTION VOLUMES FOR SAMPLE LOADING

Sample ID	Sample Concentration (cells/µL)	# of HIVE Collectors	Cells Loaded per HIVE (cells)	Loading Volume (µL)	Sample Volume (µL)	Cell Media Volume with Protein (µL)
Example	*500*	*1*	*30,000*	*1,000*	*66*	*1034*

Loading Volume *recommended at 1,000 µL per HIVE*

Cells Loaded per HIVE *recommended at 30,000 cells per HIVE (See Appendix 2)*

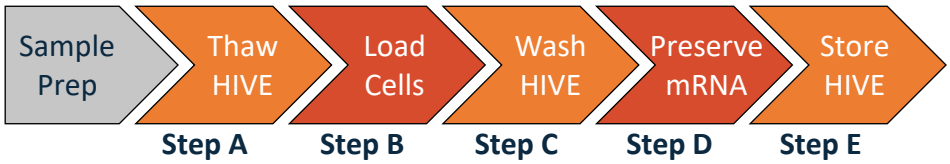
Sample Volume (µL) *= 1.1 × (# of HIVE Collectors × Cells Loaded) / Sample Concentration*

Media Volume with Protein (µL) *= (1.1 × Loading Volume) – Sample Volume*

NOTE Calculations for Sample Volume and Media Volume include a 10% excess volume for loading

CLX CAPTURE PROTOCOL

CLX SAMPLE CAPTURE WORKFLOW



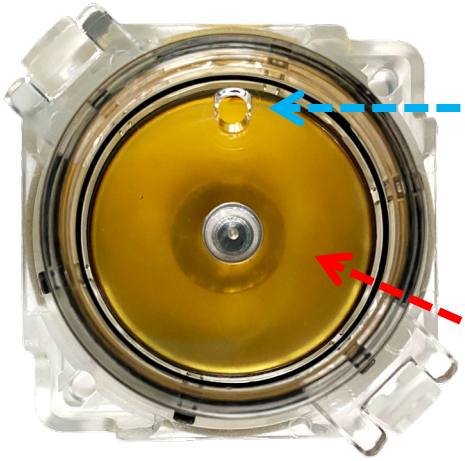
HOW TO HANDLE THE HIVE COLLECTORS

Handle the HIVE Collector along the edges of the device. Keep port at 12 o'clock position.

IMPORTANT!
Pipette along inside of the Cell Loader wall. Avoid pipetting directly onto the yellow HIVE Array.

NOTE
We recommend a vacuum aspirator to easily remove liquids and bubbles that may appear after spinning (see Troubleshooting on Page 16).

HIVE Collector

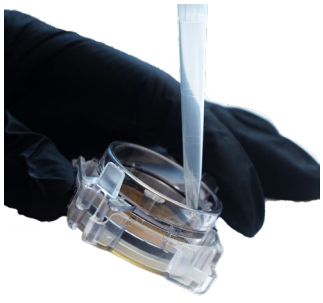


Port for Pipetting
Insert tip through port and gently pipette at an angle. Dispense along Cell Loader wall.

Yellow HIVE Array
Array of picowells pre-loaded with barcoded beads

IMPORTANT!
Always keep HIVE Collectors flat during transportation and incubation. Only tilt when directed to do so.

NOTE
Keep the port at 12 o'clock with the port edge farthest from you.

Tilt Towards You	Keep HIVE Flat	Tilt Away From You
		
When adding liquid into the HIVE Collector	When transporting and incubating	When removing liquid from the HIVE Collector

Video Protocol

NOTE

HIVE Collectors may be thawed during sample prep (see Page 6).

IMPORTANT!

Always keep HIVE Collectors flat. Only tilt when directed to do so.

IMPORTANT!

If loading samples by gravity, skip to the Alternate Step B on Page 10.

IMPORTANT!

Do Steps 5–8 one HIVE at a time!

IMPORTANT!

Cells will settle quickly. Thoroughly mix the sample immediately before any transfer.

IMPORTANT!

Do not touch HIVE Array surface! Hold the pipette tip at an angle and aspirate along the Cell Loader wall (see picture on Page 8)

NOTE

If using the recommended loading volume of 1 mL sample, dispense ~3 mL media (without added protein).

STEP A: PREPARE HIVE COLLECTORS AND SAMPLES

1. Remove HIVE Collectors from -20°C freezer.
2. Remove packaging and thaw at room temperature for **30 minutes**. Keep HIVE Collectors **flat.**
3. Label white sticker on the HIVE Collector with sample name and record HIVE serial number in the sample tracking table in Appendix 1.
4. Remove Grey Stopper from sample port and set aside for future use.

STEP B: LOAD SAMPLES BY CENTRIFUGATION

5. **Tilt the HIVE Collector away from you.** Use a pipette or vacuum aspirator to remove the thawed storage liquid (~1 mL) through the port.
6. **Tilt the HIVE Collector towards you.** Mix the sample that was prepared on Page 6 and dispense the entire Loading Volume through the port.
   - If media bubbles up through the port, remove excess media from the surface, pat the lid dry with a paper towel, and try loading again with a fresh pipette tip.
7. **Tilt the HIVE Collector towards you.** Use a P1000 pipette to dispense cell media (without added protein) through the port to fill the HIVE Collector up to 4 mL total volume.
   - **Tilt the HIVE towards you** to prevent blockages and overflow due to the large liquid volume.
8. **Place the HIVE Collector flat.** Re-insert the Grey Stopper into the sample port.
   - Some liquid may overflow when inserting the Grey Stopper. Pipet excess liquid or pat dry with a paper towel.

NOTE
Spin lid does not need to be used in this spin.

IMPORTANT!
Load Spin Plates in a radial orientation, not perpendicular (see Page 5).

NOTE
Use this Alternative Step if you do not have a suitable centrifuge.

If you use a centrifuge in Step B, DO NOT use this Alternate Step.

9. Put HIVE Collectors onto a HIVE Spin Plate. Align open corners of the HIVE Collectors with the raised pins on the HIVE Spin Plate.
10. Place loaded HIVE Spin Plates into centrifuge.
    - Balance centrifuge with additional HIVE Spin Plate and HIVE Blanks, if needed.
11. Centrifuge Spin Plates at 30 RCF for **3 minutes**. Proceed to Step 12.

ALTERNATE STEP B: LOAD SAMPLES BY GRAVITY

5Alt. **Tilt the HIVE Collector away from you.** Use a pipette or vacuum aspirator to remove the thawed storage liquid (~1 mL) through the port.

6Alt. **Tilt the HIVE Collector towards you.** Mix the sample that was prepared on Page 6 and dispense the entire sample loading volume through the port. Swirl HIVE Collector to fully cover the array surface. Re-insert the grey stopper into the sample port.

- If media bubbles up through the port, remove excess media from the surface, pat the lid dry with a paper towel, and try loading again with a fresh pipette tip.

7Alt. Place HIVE Collectors in a 4°C refrigerator. Incubate for **30 minutes**. Proceed to Step 12.

- Place all HIVE Collectors flat in the refrigerator.

IMPORTANT!
Do Steps 12–15 one HIVE at a time!

NOTE
DO NOT shake the HIVE vigorously.

NOTE
Repeat Steps 14–15 to wash twice if the cell loading media contains higher concentrations of protein (>1% FBS or >0.1% BSA).

IMPORTANT!
Do Steps 16–19 one HIVE at a time!

NOTE
Use the Blue Stopper to indicate that a HIVE Collector is loaded with cells and ready for Transcriptome Recovery.

IMPORTANT!
Always keep HIVE Collectors flat until fully frozen at -80°C or on dry ice (see Page 12).

STOP POINT

STEP C: WASHING LOADED HIVE COLLECTORS

12. Remove Grey Stopper and discard.
13. **Tilt the HIVE Collector away from you.** Remove all media by aspiration or pipetting.
    - After removing 2 mL media, **gently** shake the HIVE Collector from side-to-side to pop any large bubbles, then remove the rest of the media.
14. **Tilt the HIVE Collector towards you.** Use a P1000 pipette to dispense 2 mL CLX Sample Wash Solution through the port.
15. **Place the HIVE Collector flat.** Gently swirl the HIVE Collector to cover the entire HIVE Array.

STEP D: MOLECULAR PRESERVATION

16. **Tilt the HIVE Collector away from you.** Remove CLX Sample Wash Solution by aspiration or pipetting.
17. **Tilt the HIVE Collector towards you.** Use a P1000 pipette to dispense 2 mL CLX Cell Preservation Solution through the port.
18. **Place the HIVE Collector flat.** Gently swirl the HIVE Collector to cover the entire HIVE Array.
19. Insert the Blue Stopper into the sample port.

STEP E: STORAGE

Place loaded HIVE Collectors in the original packaging. Store frozen at **-80°C** until ready to proceed with the HIVE CLX scRNAseq Transcriptome Recovery.

- If continuing directly to Transcriptome Recovery, incubate in the Cell Preservation Solution at room temperature for at least 30 minutes first.
- If a -80°C freezer is unavailable, store at -20°C.

SHIPPING & RECEIVING

SHIPPING INSTRUCTIONS

NOTE
Ship loaded HIVE Collectors according to IATA instructions. Consult your institution's guidelines.

IMPORTANT!
HIVE Collectors will NOT be completely frozen at -20°C.

1. Place HIVE Collectors in original packaging and cover with absorbent paper towels. Tape HIVE box closed and place in a resealable plastic bag.
   - Biological Substances, Category B (UN 3373) shipments require triple waterproof packaging.
2. If stored at -20°C, freeze box for **at least 30 minutes**, either at **-80°C** or under 1–2 inches of **dry ice** in a Styrofoam box.
3. For quality control, we recommend unsealing the HIVE box and photographing the frozen HIVE Collectors before shipping.
4. Place absorbent material between the HIVE box and Styrofoam box. Use enough material to absorb all box contents.
   - Biological Substances, Category B (UN 3373) shipments require absorbent material.
5. Place HIVE box in Styrofoam box and add 30 lb dry ice for up to 3 days in shipping.
   - The HIVE Styrofoam box (16 ¾" × 16 ¾" × 15") is 85% full after adding 30 lb dry ice.
   - Do not tape or seal the Styrofoam box. The courier must be able to open Styrofoam box to replenish dry ice during shipment, if needed.
6. Place the Styrofoam box inside of a cardboard box for pickup by carrier.
   - If reusing a box, remove all markings and labels.

NOTE

For calculating volume in package, there is 2 mL per HIVE Collector.

7. Insert packing list with descriptions, volumes, and quantities.
    - Example description: “Watertight sample container with leukocytes from healthy human blood, filled with 2 mL cell preservation solution categorized as non-dangerous goods.”
8. Seal box and label outside of package:
    - Recommended labels: dry ice label, dry ice weight, red up arrows, fragile label, and “Biological Substance Category B UN3373” label.
9. Ship packages.
    - We recommend shipping overnight on a Monday to avoid weekend delays.
    - We recommend using a courier service to ensure dry-ice replenishment and correct documentation, especially for international shipments.

RECEIVING INSTRUCTIONS

1. Once received, unpackage and retrieve the box of HIVE Collectors.
2. Check that the package contains dry ice and that any temperature sensors were not activated.
3. Place the unpackaged HIVE box on dry ice.
4. Check the HIVE Collectors for Blue Stoppers. Ensure that the frozen liquid is white in color and completely covers the yellow HIVE Array surface.
5. For quality control, photograph the frozen HIVE Collectors before and after shipping.
6. Immediately put the box in -80°C storage to ensure samples remain frozen.

QUALITY CONTROL FOR SHIPPING & RECEIVING

IMPORTANT!
For quality control, photograph the frozen HIVE Collectors before and after shipping.

Good shipping

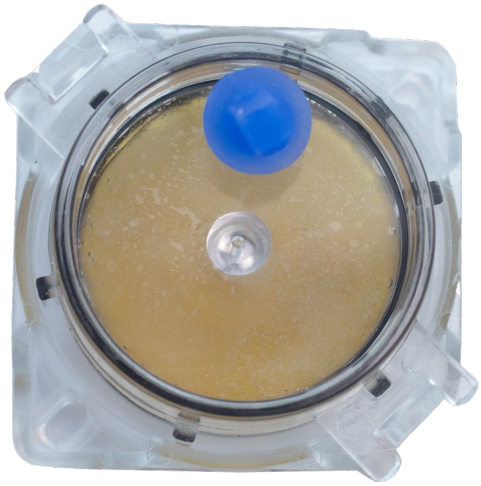
Frozen liquid is white and completely covers the HIVE Array.

Blue Stopper remains in place.

Bad shipping

Frozen liquid is white, but the liquid does not cover the right side (the yellow HIVE Array is visible). The HIVE was tilted during the freezing process.

Blue Stopper is missing.



Bad shipping

Frozen liquid is clear. CLX Cell Preservation Solution was not used.

APPENDIX 1: SAMPLE TRACKING TABLE

Sample ID	HIVE Serial #	Concentration (cells/µL)	Number cells loaded	Viability	Loading media	Experimental Condition
Example	*1437A6*	*900*	*30,000*	*93%*	*PBS+ 0.1% BSA*	*Wild Type*

APPENDIX 2: LOADING RECOMMENDATIONS

CELL RECOMMENDATIONS

The HIVE CLX scRNAseq Solution is compatible with cell inputs from 500-60,000 cells. For new users, we recommend a cell input of 30,000 cells in order to maximize cell recovery and minimize doublet formation. As the number of cells loaded per HIVE collector increases, doublet rate and sequencing cost also increase. Use the desired number of single cells recovered to determine how many cells to load, based on the table below. We recommend loading based on the number of live cells in the sample.

Single-Cell Recovery†	Cell Input	Doublet Rate†	Recommended Reads/Sample (million)‡	# of HIVEs per Novaseq SP flowcell*
220	500	0%	7	>>8
850	2,000	2%	27	>8
3,000	7,500	5%	100	8
6,000	15,000	9%	200	4
11,000	30,000	14%	400	2
17,000	60,000	36%	800	1

†Performance metrics estimated from experimental data using human PBMCs.
‡Recommendations for reads/sample balance the amount of biological information gathered with sequencing costs and should achieve 80% recovery of cells, genes, and transcripts. These are recommended starting points for most applications, but you may need to tailor sequencing depths for your specific experiments.
**The nominal NovaSeq SP flow cell offers 800 M reads.*

MEDIA RECOMMENDATIONS

We recommend using PBS + 0.1% BSA as loading media (cell media with protein) for maximum cell recovery. Use an alternative cell media if your specific sample type is incompatible with PBS + 0.1% BSA. Loading media must contain at least 1% FBS or 0.1% BSA.

APPENDIX 3: QUICK PROTOCOL FOR CAPTURE

HIVE CLX scRNAseq Sample Capture

1. Thaw HIVE Collectors for **30 minutes**.
2. During thaw, count cells and determine viability (recommended).
3. Remove thawed liquid.
4. Add 1 mL cell suspension.
5. Add 3 mL cell media (without protein). Place HIVE Collectors on Spin Plate. Spin at 30 RCF for **3 minutes**.
   - Gravity Loading: Place HIVE Collectors in 4°C refrigerator **for 30 minutes**.
6. Remove media.
7. Wash with 2 mL CLX Sample Wash Solution.
8. Add 2 mL CLX Cell Preservation Solution.
9. Insert Blue Stopper.
10. Freeze at -80°C.

TROUBLESHOOTING

Problem	Possible Causes and Suggested Solutions
The sample does not spread across the HIVE Array *When dispensed into the HIVE Collector, the sample beads on the surface and will not spread across the entire HIVE Array. The surface appears hydrophobic.*	**Make sure to dilute cells with media containing added protein** The cell suspension was most likely diluted with media without added protein. Protein (1% FBS or 0.1% BSA) helps wet the HIVE Array.
Bubbles appear after spinning *After the spin, many bubbles appear in the HIVE Collector, and they are difficult to remove with a P1000 pipette.*	**Media with high protein content creates bubbles** This is normal and will not affect cell capture. Bubble removal is easier with a vacuum aspirator than a P1000 pipette. Alternatively, use gravity loading for high protein samples.
Poor data quality after Transcriptome Recovery *Despite there being sufficient reads, the scRNAseq data contained fewer cells than expected, fewer genes than expected, and/or high mitochondrial reads.*	**Poor sample quality causes poor data quality** Poor cell viability and/or poor sample purity is the most common reason for poor data quality. We recommend using a dead cell removal kit to improve sample viability. If these sample features cannot be improved, increase the number of cells loaded per HIVE to compensate for dead and non-nucleated cells. We highly recommend the Nexcelom Cellometer® K2 Fluorescent Cell Counter for sample QC, which measures cell viability and sample purity faster and more accurately than trypan blue.[1]

REFERENCES FOR TROUBLESHOOTING

1. Honeycomb™ Appnote. Best Practices: The Cellometer® K2 for HIVE scRNAseq
https://honeycomb.bio/resources/the-cellometer-k2-for-hive-scrnaseq/?resource_id=48ab2f9b45957ab574cf005eb8a76760
2. Honeycomb™ Troubleshooting Guide
https://honeycombbio.zendesk.com/hc/en-us/articles/14881004938907-HIVE-CLX-Troubleshooting-Guide

NOTES

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MORE INFORMATION

Download protocols & example data: www.honeycomb.bio

Contact HIVE technical support: support@honeycomb.bio

Speak with a HIVE expert: sales@honeycomb.bio

Ready for quotes & ordering: NGS@revvity.com

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