CG000171 Rev B

USER GUIDE

# Chromium Single Cell DNA Training Kit

FOR USE WITH

Chromium Single Cell DNA Training Kit, 4 rxns PN-1000061

Available for use only with the indicated Chromium Controller (PN-120223;120246) or Chromium Single Cell Controller (PN-120263; 120212).

# Notices

## Document Number

CG000171 | Rev B

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### Chromium Controller Usable Temperature Range

The recommended temperature range for the Chromium Controller or the Chromium Single Cell Controller when running the protocol outlined in this Chromium Single Cell DNA Reagents Kits User Guide is 19-25°C (66-77°F), which is different than the usable temperature range of 18-28°C (64-82°F) stated in the Chromium Controller Specifications (Document CG00020) and the Chromium Single Cell Controller Specifications (Document CG00050). Running the Chromium Controller or the Chromium Single Cell Controller outside the recommended temperature range of 19-25°C (66-77°F) when using the reagent kits and chip kits described herein will invalidate the warranty of these products.

## Support

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# Table of Contents

# Introduction

Objective

Chromium Single Cell DNA Training Kit

Chromium Single Cell DNA Accessories

Chromium Accessories

Recommended Thermal Cyclers

Additional Kits, Reagents & Equipment

## Objective

The purpose of this User Guide is to train new users on:

- How to handle Activation Agent and Cell Matrix
- How to prepare Cell Bead Mix
- How to load a Chromium Chip C with the Cell Bead Mix, CB Polymer, and Partitioning Oil
- How to load a Chromium Chip C into the Chromium Controller and run the Controller
- How to inspect the resulting Cell Beads in the Chromium Chip C
- How to transfer the Cell Beads
- How to process the Cell Beads in preparation for loading Chromium Chip D

Firmware Version 3.0 or higher is required in the Chromium Controller or the Single Cell Chromium Controller used for this protocol.

For guidance on other items, refer to the User Guides cited below:

- For guidance on qualifying the Chromium Controller or Chromium Single Cell Controller, refer to the Chromium Controller Specifications (CG00020) or the Chromium Single Cell Controller Specifications (CG00050), and the Chromium Controller Readiness Test (CG00025) or the Chromium Single Cell Controller (CG00051).
- For guidance on preparing a sample that can be quantified and moved through library generation and sequencing, refer to the applicable Sample Preparation Demonstrated Protocols and to the Chromium Single Cell DNA Reagent Kits User Guide (CG000153).

# Chromium Single Cell DNA Training Kit

## Chromium Single Cell DNA Training Kit, 4 rxns PN-1000061

### Chromium Single Cell DNA Cell Bead Reagent Kit, 4 rxns PN-1000034 (store at –20°C)

**Chromium**
Single Cell DNA
Cell Bead Reagents

| | # | PN |
|---|---|---|
| Activation Agent (4 rxns) | 1 | 2000039 |
| CB Buffer | 2 | 2000040 |

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### Chromium Single Cell DNA Cell Bead Polymer Kit, 4 rxns PN-1000035 (store at 4°C)

**Chromium**
Single Cell DNA
Cell Bead Polymer

| | # | PN |
|---|---|---|
| CB Polymer | 1 | 2000041 |
| Cell Matrix (4 rxns) | 1 | 2000042 |
| Lysis 1 | 1 | 2000049 |
| Lysis 2 | 1 | 3000146 |

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### Chromium Chip C Single Cell DNA Kit, 16 rxns PN-1000032 (store at ambient temperature)

**Chromium**
Partitioning Oil

| | # | PN |
|---|---|---|
| Partitioning Oil | 2 | 220088 |

**Chromium**
Recovery Agent

| | # | PN |
|---|---|---|
| Recovery Agent | 2 | 220016 |

**Chromium**
Chip C & Gaskets

| | # | PN |
|---|---|---|
| Chip C Single Cell DNA | 2 | 2000030 |
| Gasket | 1 | 3000072 |

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## Flowmi™ Filters, 50 rxns, PN-1000055

| | # | PN |
|---|---|---|
| Flowmi | 1 | 1000055 |

## Chromium Single Cell DNA Accessory Kit, PN-1000058

| Product | # | PN (Orderable) | PN (Item) |
|---|---|---|---|
| 10x Magnetic Separator A | 1 | 1000054* | 2000067 |
| 10x Chromium Chip D Holder | 1 | 1000053* | 3000109 |
| CABLE USB A MALE - B MALE 1M BLK | 1 | - | 3000173 |

*May be ordered individually or as part of the Chromium Single Cell DNA Accessory Kit. Also available as part of the Chromium Controller Accessory Kit, PN-110204.

## Chromium Accessories

| Product | PN (Orderable) | PN (Item) |
|---|---|---|
| 10x Vortex Adapter | 120251 | 330002 |
| 10x Vortex Clip | 120253 | 230002 |
| 10x Chip Holder | 120252 | 330019 |

## Recommended Thermal Cyclers

Thermal cyclers used must support uniform heating of 100 µl emulsion volumes.

| Supplier | Description | Part Number |
|---|---|---|
| BioRad | C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module | 1851197 |
| Eppendorf | MasterCycler Pro | North America 950030010<br>International 6321 000.01 |
| Thermo Fisher Scientific | Veriti 96-Well Thermal Cycler | 4375786 |

## Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Chromium Single Cell DNA protocol. Substituting materials may adversely affect system performance.

| Supplier | Description | Part Number (US) |
|---|---|---|
| **Plastics** | | |
| Eppendorf | PCR Tubes 0.2 ml 8-tube strips<br>DNA LoBind Tubes, 1.5 ml<br>DNA LoBind Tubes, 2.0 ml | 951010022<br>022431021<br>022431048 |
| USA Scientific | TempAssure PCR 8-tube strip | 1402-4700 |
| Rainin | Tips LTS W-O 200UL Filter RT-L200WFLR<br>Tips LTS 20UL Filter RT-L10FLR<br>Tips LTS 200UL Filter RT-L200FLR<br>Tips LTS 1ML Filter RT-L1000FLR | 30389241<br>30389226<br>30389240<br>30389213 |
| **Kits & Reagents** | | |
| Thermo Fisher Scientific | Nuclease-free Water | AM9937 |
| Corning Cellgro | Phosphate-Buffered Saline (PBS) 1X without calcium and magnesium | 21-040-CV |
| Millipore Sigma | 1 N NaOH | S2770-100ML |
| | Phosphate-Buffered Saline (PBS) with 10% Bovine Albumin | SRE0036 |
| Ricca Chemical Company | Glycerin (glycerol), 50% (v/v) Aqueous Solution | 3290-32 |
| Teknova | 1 M Tris-HCl, pH 7.2 | T1072 |
| **Equipment** | | |
| VWR | Vortex Mixer<br>Divided Polystyrene Reservoirs | 10153-838<br>41428-958 |
| Eppendorf | Eppendorf ThermoMixer C<br>Eppendorf SmartBlock PCR 96<br>Eppendorf SmartBlock 1.5 ml, Thermoblock for 24 Reaction Vessels | 5382000023<br>5306000006<br>5360000038 |
| Rainin | Pipet-Lite LTS Pipette L-2XLS<br>Pipet-Lite LTS Pipette L-10XLS<br>Pipet-Lite LTS Pipette L-20XLS<br>Pipet-Lite LTS Pipette L-100XLS<br>Pipet-Lite LTS Pipette L-200XLS<br>Pipet-Lite LTS Pipette L-1000XLS<br>Pipet-Lite Multi Pipette L8-10XLS<br>Pipet-Lite Multi Pipette L8-20XLS<br>Pipet-Lite Multi Pipette L8-50XLS<br>Pipet-Lite Multi Pipette L8-200XLS | 17014393<br>17014388<br>17014392<br>17014384<br>17014391<br>17014382<br>17013802<br>17013803<br>17013804<br>17013805 |

Choose either Eppendorf or USA Scientific PCR 8-tube strips.

# Tips & Best Practices

## Icons


Tip & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

## Images

- The Training Kit is for 4 rxns. However, all images and illustrations shown are for 8 rxns.

## Emulsion-safe Plastics

- Use 10x Genomics validated emulsion-safe plastic consumables when handling GEMs as some plastics can destabilize GEMs.

## General Reagent Handling

- Fully thaw and thoroughly mix reagents before use.
- Calculate reagent volumes with 10% excess of 1 rxn values.
- Cover Partitioning Oil tubes and reservoirs to minimize evaporation.

## 50% Glycerol Solution

- Purchase 50% glycerol solution from Ricca Chemical Company, Glycerin (glycerol), 50% (v/v) Aqueous Solution, PN-3290-32.

  OR

- Prepare 50% glycerol solution:

  i. Mix an equal volume of water and 99% Glycerol, Molecular Biology Grade.

  ii. Filter through a 0.2-µm filter.

  iii. Store at –20°C in 1-ml LoBind tubes. 50% glycerol solution should be equilibrated to room temperature before use.

## Pipette Calibration

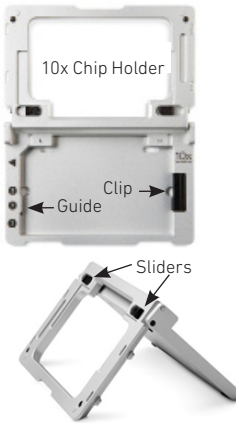
- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is **critical** in **Training Step 2**.

## Chromium Chip Handling

- Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, and dusty surfaces.
- Execute steps without pause or delay, unless indicated. When multiple chips are to be used, load, run, and collect the content from one chip before loading the next.
- Fill all unused input wells in rows labeled 1, 2, and 3 on a chip with an appropriate volume of 50% glycerol solution before loading the used wells. DO NOT add glycerol to the Recovery Wells.
- Avoid contacting the bottom surface of the chip with gloved hands and other surfaces. Frictional charging can lead to inadequate priming of the channels, potentially leading to either clogs or wetting failures.
- Minimize the distance that a loaded chip is moved to reach the Chromium Controller.
- Keep the chip horizontal to prevent wetting the gasket with oil, which depletes the input volume and may adversely affect the quality of the assay.

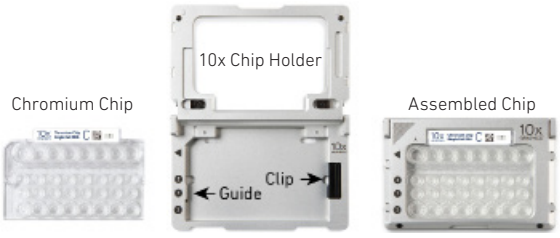
## 10x Chip Holders

- 10x Chip Holders encase Chromium Chips.
- The holder lid flips over to become a stand, holding the chip at 45 degrees for optimal Recovery Well content removal.
- Squeeze the black sliders on the back side of the holder together to unlock the lid and return the holder to a flat position.
- Use the 10x Chip Holder to encase Chip C.




## Chromium Chip & Holder Assembly

- Align notch on the chip (upper left corner) and the holder.
- Insert the left-hand side of the chip under the guide. Depress the right-hand side of the chip until the spring-loaded clip engages.
- Close the lid before dispensing reagents into the wells.



## Activation Agent Handling

- Use one tube of Activation Agent per sample. DO NOT puncture foil seal of tubes not used at the time.
- Store unused tubes at –20°C.

## Cell Matrix Handling

- Use one tube of Cell Matrix per sample. DO NOT puncture foil seal of tubes not used at the time.
- Store unused tubes at 4°C. DO NOT freeze Cell Matrix.

## Chromium Chip C Loading

- Place the assembled chip and holder flat on the bench with the lid closed.
- Dispense at the bottom of the wells without introducing bubbles.
- Wait for the Cell Bead Mix to drain into the bottom of the pipette tips and dispense again to ensure complete volume transfer.
- Refer to Load Chromium Chip C for specific instructions.


## 10x Magnetic Separator A

- Offers one position of the magnets relative to a tube.
- 10x Magnetic Separator **A** is different from 10x Magnetic Separator.
- Use for Cell Bead Processing.

# Training Step 1

## Cell Bead Generation

**1.1** Load Chromium Chip C

**1.2** Run the Chromium Controller & Transfer Cell Beads

1

## 1.0 Cell Bead Generation

| GET STARTED! | | | | |
|---|---|---|---|---|
| **Action** | **Item** | **10x PN** | **Preparation & Handling** | **Storage** |
| **Equilibrate to Room Temperature** | **1X PBS** | - | - | - |
| **Place on Ice** | **Activation Agent** (Cell Bead Reagent Kit) | 2000039 | Use one tube per sample. Puncture the tube foil seal immediately before use. Return unused tubes to –20°C.  | –20°C |
| | **Cell Matrix** (Cell Bead Polymer Kit) | 2000042 | Use one tube per sample. Puncture the tube foil seal immediately before use. Return unused tubes to 4°C.  | 4°C |
| | **CB Polymer** (Cell Bead Polymer Kit) | 2000041 | - | 4°C |
| | **Training Sample** PBS + 0.04% BSA | - | Training sample (PBS + 0.04% BSA) is used as a substitute for single cell suspension, which is prepared in PBS + 0.04%. BSA | - |
| **Obtain** | **Partitioning Oil** | 220088 | - | Ambient |
| | **Nuclease-free Water** | - | - | - |
| | **Chromium Chip C** | 2000030 | See Tips & Best Practices. | Ambient |
| | **10x Vortex Adapter** | 330002 | See Tips & Best Practices. | Ambient |
| | **10x Gasket** | 370017 | See Tips & Best Practices. | Ambient |
| | **10x Chip Holder** | 330019 | See Tips & Best Practices. | Ambient |
| | **50% glycerol solution** If using <8 reactions | - | See Tips & Best Practices. | Ambient |

Firmware Version 3.0 or higher is required in the Chromium Controller or the Single Cell Chromium Controller used for the Single Cell DNA protocol.

## 1.1 Load Chromium Chip C

See Tips & Best Practices for chip handling instructions. When loading the chip, raising and depressing the pipette plunger should each take **~5 sec**. When dispensing, raise the pipette tips at the same rate as the liquid is rising, keeping the tips slightly submerged.

TIPS

**a. Assemble Chromium Chip C in a 10x Chip Holder** See Tips & Best Practices

**b. Dispense 50% Glycerol Solution into Unused Chip Wells** (if <8 samples per chip)

i. **75 µl** into unused wells in **row labeled 1**.

ii. **40 µl** into unused wells in **row labeled 2**.

iii. **200 µl** into unused wells in **row labeled 3**.

DO NOT add 50% glycerol solution to the top row of Recovery Wells.
DO NOT use any substitute for 50% glycerol solution.

**c. Prepare Cell Bead Mix**

i. Remove the Activation Agent and Cell Matrix tube strips from the holders. Centrifuge briefly. Place on ice.

ii. Using a pipette tip, puncture the foil seal of one Activation Agent and one Cell Matrix tube per sample and enlarge the holes, avoiding contact between the pipette tip and the Activation Agent or the Cell Matrix.

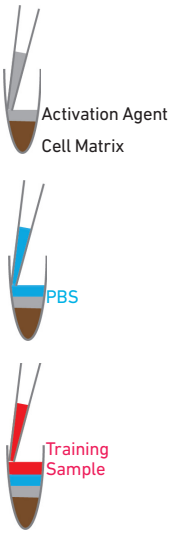
iii. Add **20 µl** nuclease-free water to each Activation Agent tube. Pipette mix to dissolve.

DO NOT mix during steps iv-vi.

iv. Layer **4.7 µl** Activation Agent solution on top of the Cell Matrix avoiding contact between the Cell Matrix and the pipette tip.
DO NOT mix.

v. Layer the **6.5 µl** PBS on top of the Activation Agent. DO NOT mix.

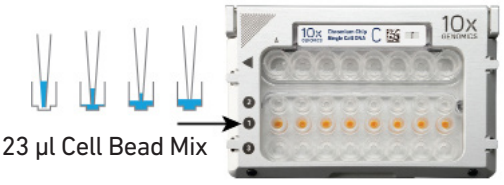
vi. Resuspend training sample and **immediately** layer **4.0 µl** training sample on top of PBS.
DO NOT mix.



See Tips & Best Practices for chip handling instructions. When loading the chip, raising and depressing the pipette plunger should each take **~5 sec**. When dispensing, raise the pipette tips at the same rate as the liquid is rising, keeping the tips slightly submerged.

**d. Load Row Labeled 1**

Using a **50-µl multi-channel pipette** (set to 20 µl), gently pipette mix the Cell Bead Mix until homogeneous. Using the same pipette tips, dispense **23 µl** Cell Bead Mix into the bottom center of each well in the **row labeled 1** without introducing bubbles.
Wait for the Cell Bead Mix to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.




Dislodge any Cell Bead Mix stuck along the sidewalls of the well using a pipette tip. Accurate input volume is critical for correct Cell Bead volume yield and optimal performance.

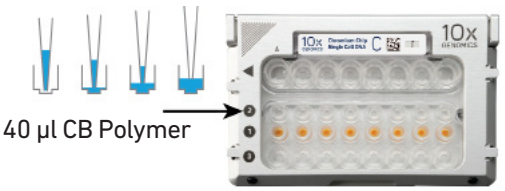
**e. Prepare CB Polymer**

Centrifuge the CB Polymer briefly. Vortex **10 sec**. Centrifuge briefly again.

**f. Load Row Labeled 2**

Using a **single-channel** pipette, slowly dispense **40 µl** CB Polymer into the bottom center of each well in the **row labeled 2** without introducing bubbles.

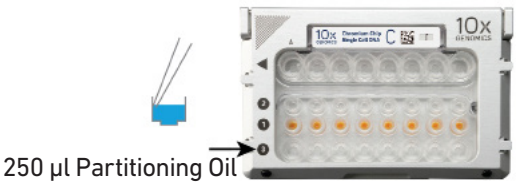
Wait **60 sec** for the chip to prime.




**g. Load Row Labeled 3**

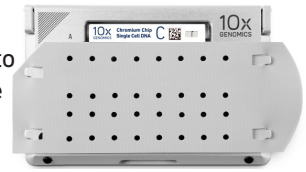
Dispense **250 µl** Partitioning Oil into each well in the **row labeled 3.**

Failure to add Partitioning Oil can damage the Chromium Controller.




**h. Attach 10x Gasket**

Align the notch with the top left-hand corner. Ensure the gasket holes are aligned with the wells. Avoid touching the smooth gasket surface. DO NOT press down on the gasket.



## 1.2 Run the Chromium Controller & Transfer Cell Beads

**a.** Press the eject button on the Controller to eject the tray.

**b.** Place the loaded chip with the gasket in the tray. Press the button to retract the tray.

**c.** Confirm program on screen. Press the play button.

**d.** At completion of the run **(~11.5 min)**, the Controller will chime. **Immediately** proceed to the next step. Steps e – i should be executed within **5 min**.

**e.** Press the eject button to remove the chip.

**f.** Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees.

**g.** Remove **60 µl** Partitioning Oil from lowest point of the Recovery Wells in the top row using **regular-bore** pipette tips.

**h.** Using **wide-bore** pipette tips (pipette set to 95 µl), over the course of **~30 sec** aspirate remaining Partitioning Oil and Cell Beads from the bottom of each Recovery Well and transfer into a PCR 8-tube strip. Dispense slowly with the pipette tips against the sidewalls without introducing bubbles. Using the same pipette tips, transfer any remaining Partitioning Oil and Cell Beads from the Recovery Wells to the tubes.

DO NOT flick, shake or vortex the tubes to prevent wetting the sidewalls and caps of the tubes.

**i.** **Immediately** place the capped tube strip on a thermomixer and shake at **21°C** for **16-24 h** at **1000 rpm.**

Usable Temperature Range
19-25°C (66-77°F)

Expose Wells

Remove Partitioning Oil

Transfer Cell Beads
(use wide-bore pipette tips)

Cell Beads (wide-bore pipette tips)

# Training Step 2

## Cell Bead Processing

**2.1** Solvent Exchange

**2.2** Lysis

**2.3** Filtration

## 2.0 Cell Bead Processing

| GET STARTED! | | | | |
|---|---|---|---|---|
| Action | Item | 10x PN | Preparation & Handling | Storage |
| **Equilibrate to Room Temperature** | **1X PBS** | - | - | - |
| | **CB Buffer** (Cell Bead Reagent Kit) | 2000040 | - | –20°C |
| **Place on Ice** | **Lysis 1** (Cell Bead Polymer Kit) | 2000049 | Mix by inverting tube 5x. DO NOT vortex. | 4°C |
| | **Lysis 2** (Cell Bead Polymer Kit) | 3000146 | Vortex. Centrifuge briefly. | 4°C |
| **Obtain** | **Recovery Agent** | 220016 | - | Ambient |
| | **0.4 N NaOH** Prepare 1 ml for 8 reactions | - | Prepare fresh. | Ambient |
| | **1 M Tris-HCl, pH 7.2** | - | - | Ambient |
| | **10x Magnetic Separator A** | 2000067 | Different from the 10x Magnetic Separator. | Ambient |
| | **10x Vortex Clip** | 230002 | - | Ambient |
| | **1.5 ml magnetic tube holder** | - | - | Ambient |
| | **Flowmi Filters, 70 µm** | 1000055 | - | Ambient |

## 2.1 Solvent Exchange

**a.** Remove the tube strip containing Cell Beads from the thermomixer.

**b.** Remove **45 µl** Partitioning Oil from the bottom of the tube. **5-10 µl** Partitioning Oil should remain. DO NOT aspirate Cell Beads. If Cell Beads are aspirated, return the solution to the tube, reduce removal volume by **5 µl**, and reattempt removal.

**c.** Add **95 µl** PBS.

**d.** Cap the tube strip and place in a 10x Vortex Clip. Vortex **20 sec**. Centrifuge **~1 sec** only. The Cell Bead suspension may not be uniform.

**e.** Add **60 µl** Recovery Agent. Wait **60 sec**.

**f.** Gently invert ~25x. DO NOT vortex.

**g.** Centrifuge **10 sec** to separate the phases. The top aqueous layer contains Cell Beads. The bottom pink layer contains Recovery Agent/Partitioning Oil. Place on a 10x Magnetic Separator **A** for **30 sec.**

TIPS

10x Magnetic Separator **A** (magnet **A**) is different from 10x Magnetic Separator.

**h.** Insert pipette tips along the sidewalls of the tube, away from the Cell Beads and slowly remove **120 µl** Recovery Agent/ Partitioning Oil/PBS from the bottom of the tube, retaining the Cell Beads. If the level of samples remaining is not at the etched mark on magnet **A**, adjust by either adding or removing PBS.

A small volume of Recovery Agent/ Partitioning Oil may remain in the tube. DO NOT aspirate Cell Beads.

**i.** Add **120 µl** PBS. Remove from magnet **A**.

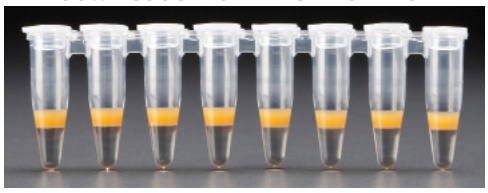
**j.** Gently invert until homogeneous and centrifuge **~1 sec**.

**k.** Place on magnet **A** for **30 sec**.

**l.** Remove **120 µl** from the bottom of the tube, retaining the Cell Beads.

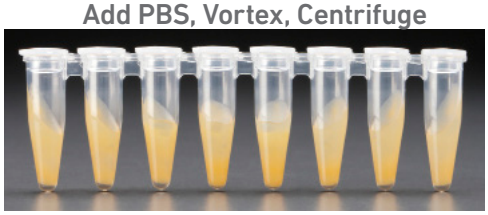
**m.** Remove from magnet **A**.

Cell Beads from Thermomixer

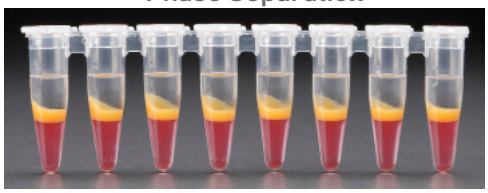


Remove Oil (~5-10 µl Oil Remains)

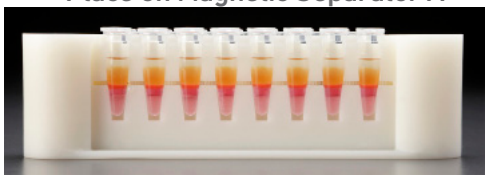
Add PBS, Vortex, Centrifuge



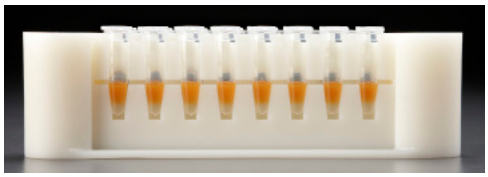
Phase Separation



Place on Magnetic Separator A



Retain Cell Beads



## 2.2 Lysis

**a.** Add **120 µl** Lysis 1. Mix by inverting until homogeneous. Centrifuge **~1 sec**.

**b.** Place on magnet **A** for **30 sec**.

**c.** Remove **120 µl** from the bottom of the tube, retaining the Cell Beads.

**d.** Incubate in a thermal cycler using the following protocol.

| Lid Temperature | Reaction Volume | Run Time |
|---|---|---|
| 85°C | 75 µl | 10 min |
| **Step** | **Temperature** | **Time** |
| Incubate | 72°C | 00:10:00 |
| Hold | 4°C | Hold |

**e.** Add **100 µl** Lysis 1. Mix by inverting until homogeneous. Centrifuge **~1 sec**.

**f.** Add **5 µl** Lysis 2. **Immediately** mix by inverting until homogeneous.

**g.** Incubate **15 min** at **room temperature**. Gently invert every **5 min** to prevent settling of beads.

**h.** Centrifuge **~1 sec**. Place on magnet **A.** Wait **30 sec**.

**i.** Remove **105 µl** from the bottom of the tube, retaining the Cell Beads.

**j.** Add **120 µl** PBS. Mix by inverting until homogeneous. Centrifuge **~1 sec**.

**k.** Place on magnet **A.** Wait **30 sec**.

**l.** Remove **120 µl** from the bottom of the tube, retaining the Cell Beads.

**m.** **Repeat** steps j – l for a total of 2 washes.

**n.** Adjust the liquid level such that the meniscus is at the etched mark on magnet **A** by either adding or removing PBS.

**o.** Remove from magnet **A.** Add **75 µl** freshly prepared 0.4 N NaOH. Mix by inverting until homogeneous.

**p.** Incubate **5 min** at **room temperature.** Gently invert every **~1 min.** Centrifuge **~1 sec** at the end of incubation.

**q.** Add **50 µl** 1 M Tris-HCl, pH 7.2. Mix by inverting until homogeneous. Centrifuge **~1 sec**.

**r.** Place on magnet **A.** Wait **30 sec**.

**s.** Remove **120 µl** from the bottom of the tube, retaining the Cell Beads.

**t.** Add **120 µl** CB Buffer. Mix by inverting until homogeneous. Centrifuge **~1 sec**.

**u.** Place on magnet **A**. Wait **30 sec**.

**v.** Remove **120 µl** from the bottom of the tube, retaining the Cell Beads.

**w.** **Repeat** steps t – v for a total of 2 washes.

## 2.3 Filtration

**a.** Add **450 µl** CB Buffer to a 1.5-ml microcentrifuge tube for each sample.

**b.** Using a **single-channel** pipette (set to 75 µl), pipette mix Cell Beads. Using the same pipette tip, transfer **all** Cell Beads to the microcentrifuge tube containing CB Buffer.

**c.** Using a **single-channel** pipette (set to 520 µl), gently pipette mix the Cell Bead suspension (volume may be less than **520 µl**). Using the same pipette tip, aspirate the Cell Bead suspension and attach the pipette tip to a **70 µm** Flowmi filter.

**Attach Flowmi Filter**

**d.** **Immediately** pass the Cell Bead suspension slowly through the Flowmi filter to the **first stop**, into a new 1.5-ml microcentrifuge tube. Variation in volume of filtrate is normal.

**Pass through Flowmi Filter**

**e.** Place the capped tube containing the Cell Bead suspension on a 1.5-ml magnetic tube holder for **3 min** or until the solution clears.

**f.** Remove **260 µl** solution, retaining the Cell Beads.

**g.** Remove from the magnet. Pipette mix using a **200-µl single-channel pipette** (set to 185 µl) until homogeneous without introducing bubbles. Using the same pipette tip, transfer exactly **185 µl** to a new tube strip. Some Cell Beads may remain in the 1.5-ml microcentrifuge tube. If volume is less than **185 µl,** add CB buffer from the CB Buffer bottle to bring volume to **185 µl**.

**Remove 159 µl**

**h.** Centrifuge **~1 sec**. Place on magnet **A** for **3 min**.

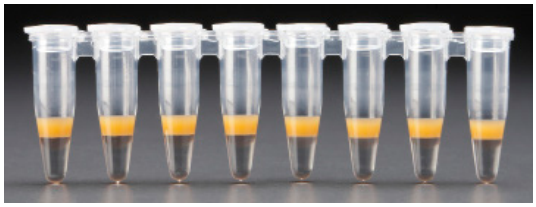
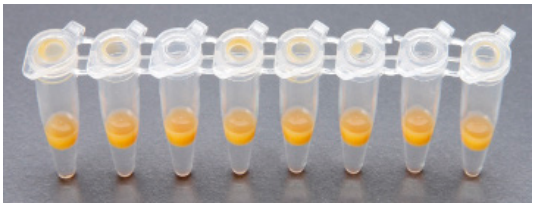
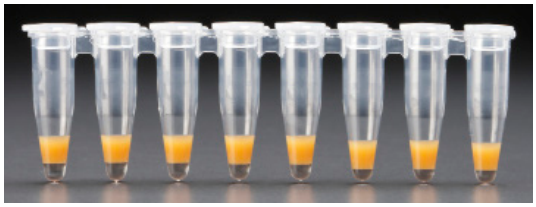
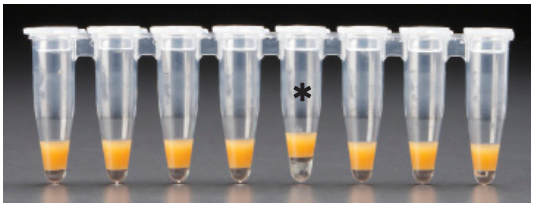
**i.** Using a **200-µl single-channel pipette**, slowly remove **159 µl** from the bottom of the tube. A variable small volume of Cell Beads may be aspirated.

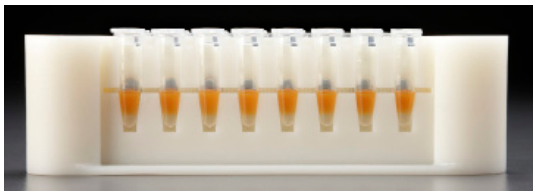
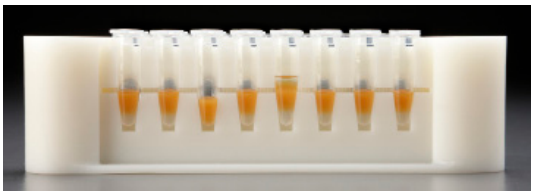
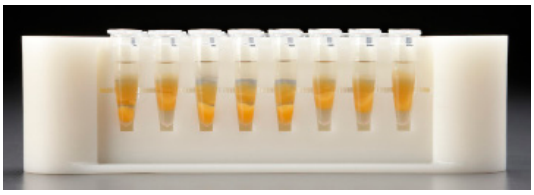
**j.** Using a **single-channel pipette,** add **7 µl** CB Buffer from the CB Buffer bottle to the retained Cell Beads. Remove from the magnet and place on ice.

# Troubleshooting

# 7.0 Troubleshooting

## 7.1 Cell Beads

| STEP | NORMAL | PROBLEM & SOLUTION |
|---|---|---|
| 1.1 d<br>Mixing the Cell Bead Mix | Cell Bead Mix is uniform with no visible magnetic bead clumps. | Cell Bead Mix has magnetic bead clumps.<br><br>**Solution:** Using a pipette tip, aspirate from the bottom of the tube and dispense at the liquid surface multiple times to break the magnetic bead clumps. |
| 1.1 d<br>Loading Cell Bead Mix into Chip C | Dispense **23 µl** Cell Bead Mix into the bottom center of each well in **row labeled 1** without introducing bubbles. | **<23 µl** Cell Bead Mix aspirated in the pipette tip for dispensing.<br><br>**Solution:** Using a pipette tip, push down Cell Bead Mix stuck to the sidewalls of the tube. Slowly aspirate from the bottom of the tube holding the pipette tips in the solution for **~2 sec** after the plunger is fully released. |
| 1.2 i<br>Transferring tubes to the thermomixer |  <br>Cell Beads are uniform and not stuck on the sidewalls or caps of the tubes. |  <br>Cell Beads are stuck on the sidewalls and caps of the tubes.<br><br>DO NOT flick, shake or vortex the tubes when transferring to the thermomixer.<br>**Solution:** Centrifuge briefly to recover beads. |
| 2.1 b<br>After removal of Partitioning Oil |  <br>**5-10 µl** Partitioning Oil remaining in the tubes. |  <br>**>10 µl** Partitioning Oil remaining in tube*.<br><br>**Solution:** Remove oil from the tube* until remaining oil volume is **5-10 µl**. |

| STEP | NORMAL | PROBLEM & SOLUTION |
|---|---|---|
| 2.2 n<br>Removal of PBS from the bottom of the tubes, retaining the Cell Beads |  <br>PBS removed uniformly, retaining Cell Beads. |  <br>Variable removal of PBS.<br><br>**Solution:** Add or remove PBS until uniform. |
| 2.2 s<br>Cell Beads migrating to the magnets |  <br>Cell Beads migrate to the magnets uniformly. |  <br>Cell Beads migrate to the magnets variably.<br><br>**Solution:** Use pipette tip to gently mix Cell Beads to enable uniform migration to the magnets.<br><br>  |
| 2.3 d<br>Pass Cell Bead suspension through Flowmi filter | Cell Bead suspension passes through the Flowmi filter into a new 1.5-ml microcentrifuge tube. | Flowmi filter clogs while passing the Cell Bead suspension.<br><br>**Solution:** Discard clogged Flowmi filter and proceed with a new Flowmi filter. Some loss in Cell Bead suspension volume is expected. |

## 7.2 Chromium Controller Errors

If the Chromium Controller or the Chromium Single Cell Controller fails to start, an error tone will sound and one of the following error messages will be displayed:

a. **Chip not read – Try again:** Eject the tray, remove and/or reposition the 10x Chip Holder assembly and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

b. **Check gasket:** Eject the tray by pressing the eject button to check there is a 10x Gasket on the Chromium Chip. In the case when the 10x Gasket installation was forgotten, install and try again. In the case when a 10x Gasket was already installed, remove, reapply, and try again. If the error message is still received after trying either of these more than twice, contact support@10xgenomics.com for further assistance.

c. **Pressure not at Setpoint:**

   i. If this message is received within a few seconds of starting a run, eject the tray by pressing the eject button and check for dirt or deposits on the 10x Gasket. If dirt is observed, replace with a new 10x Gasket and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

   ii. If this message is received after a few minutes into the run, the Chromium Chip must be discarded. **Do not try running this Chromium Chip again as this may damage the Chromium Controller.**

d. **CAUTION: Chip Holder not Present:** Eject the tray by pressing the eject button to check there is a 10x Chip Holder encasing the Chromium Chip. In the case when the 10x Chip Holder was forgotten, install with a 10x Gasket in place, and try again. If the error message is still received after a 10x Chip Holder is confirmed as in place, contact support@10xgenomics.com for further assistance.

e. **Invalid Chip CRC Value:** This indicates the Chromium Chip has encountered an error, should not be run, and must be discarded. Contact support@10xgenomics.com for further assistance.

f. **Endpoint Reached Early:**
If this message is received, contact support@10xgenomics.com for further assistance.