User Manual

Version 1.0 - UMFC4501INT

Evercode™ Cell and Nuclei Fixation v4 with INTEGRA ASSIST PLUS

For use with

ECFC4501

ECFN4501

Support Suite

Legal Notices

This document and its contents are proprietary to Parse Biosciences, Inc. (“Parse Biosciences”) and are intended solely for use by its customers in connection with the use of the product(s) described herein and for no other purpose. The products may be used solely FOR RESEARCH PURPOSES, AND MAY NOT BE USED IN ANY DIAGNOSTIC OR THERAPEUTIC USE IN HUMANS OR ANIMALS. This document and its contents shall not be used or distributed for any other purpose and/ or otherwise communicated, disclosed or reproduced in any way whatsoever without the prior written consent of Parse Biosciences.

No rights are granted under this document with respect to any of Parse Biosciences’ intellectual property rights. The license to use of any products described herein is subject to a separate written agreement between Parse Biosciences and the applicable user.

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. Parse Biosciences shall have no liability for any direct, indirect, consequential or incidental damages arising out of any failure to use the product(s) in strict compliance with the terms herein.

This document may contain references to third-party sources of information, hardware or software, products, or services and/ or third-party web sites (collectively “Third Party Information”). Parse Biosciences does not control, and is not responsible for, any Third Party Information. The inclusion of Third Party Information in this document does not imply endorsement by Parse Biosciences of the Third Party Information or the third party in any way.

The product(s) described in this document are provided for one-time use by the purchaser and may not be re-used, refurbished or resold. In addition, such product(s) may not be altered, changed or modified by anyone other than Parse Biosciences and its authorized agents, and Parse Biosciences will not be liable for any such alterations, changes or modifications.

Patents pending in the U.S. and other countries.

Patent information about the product(s) described herein can be found at:
https://www.parsebiosciences.com/patents/

Table of Contents

Overview

Workflow

The Evercode Cells and Nuclei Fixation workflow is compatible with the INTEGRA ASSIST PLUS to enable the large-scale fixation of single cell/nuclei through a robust, semi-automated process.

From a single cell/nuclei suspension, the Evercode Cell Fixation and Nuclei Fixation kits generate fixed and permeabilized cells/nuclei ready for use in all downstream Evercode assays.

Two different throughput options provide the flexibility to process up to 96 samples in parallel:

- 48 Reactions workflows uses half of ECFC4501/ECFN4501
- 96 Reactions workflows uses ECFC4501/ECFN4501

Fixation maintains cell structure, prevents RNA degradation, and locks the RNA inside the cells, which are crucial for downstream processing with Evercode split-pool combinatorial barcoding technology (Figures 1 and 2).

Because fixed samples are also stable for up to 6 months at -80°C, Evercode Cell/Nuclei Fixation Kit with INTEGRA ASSIST PLUS provides flexibility by separating sample collection from library preparation. It also enables samples to be stored and batched after fixation so they can be processed through library preparation together, reducing the potential of batch effects.

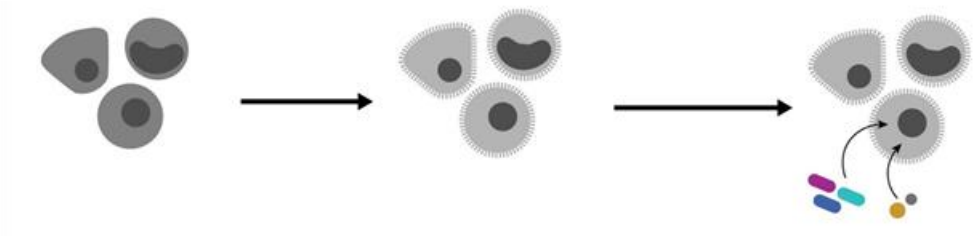


Figure 1: Evercode Cell Fixation. Cells in suspension are fixed and permeabilized before undergoing the split-pool combinatorial barcoding steps.

Figure 2: Evercode Nuclei Fixation. Nuclei in suspension are fixed and permeabilized before undergoing the split-pool combinatorial barcoding steps.

Important Guidelines

These guidelines provide additional information to obtain optimal performance beyond the detailed instructions in the protocol. For additional questions not discussed below, please contact us at support@parsebiosciences.com. We also have a library of additional resources and videos on our support site at https://support.parsebiosciences.com/.

Sample Input

- This protocol begins with a previously prepared single cell/nuclei suspension. We recommend suspensions with >70% viability (ideally above 90%) and <5% aggregation/debris.
- If cells/nuclei were previously frozen, ensure the suspension is completely thawed and in suspension before beginning fixation.
- We recommend minimizing the length of time samples are stored on ice prior to fixation, as it can negatively impact results.
- Between 100,000 and 1 million cells/nuclei can be fixed in a single reaction. However, we recommend using the highest number available up to 1 million total. Exceeding 1 million cells/nuclei in a single fixation will result in substantially elevated doublet rates.
- The minimum input into fixation should also be determined based on how the samples will be processed downstream. The table below provides guidance on the post-fixation concentrations needed for downstream kits. However, more or less sample input may be required depending on the exact experimental design. To accurately determine required post-fixation cell/nuclei concentrations and volumes, reference the relevant Sample Loading Table.
- Note that retention during fixation varies typically between 40-60%, and some cells/nuclei will be lost when freezing and thawing fixed samples, typically between 5-15%. The final concentration of cells/nuclei post-fixation is also influenced by the resuspension volume. These factors should all be taken into account when determining how much sample input is needed for fixation.

CELL/NUCLEI CONCENTRATIONS		
Kit	Target Post-Fixation Concentration/µL	Minimum Post-Thaw Concentration to Fully Load Kit/µL
Evercode WT	≥ 1,000 cells/nuclei	520 cells/nuclei
Evercode WT Mega	≥ 3,000 cells/nuclei	2,126 cells/nuclei

Avoiding RNase Contamination

- Standard precautions should be taken to avoid introducing RNases into samples or reagents throughout the workflow. Always wear proper laboratory gloves and use aseptic technique.
- ·Although RNases are not inactivated by ethanol or isopropanol, they are inactivated by products such as RNaseZap RNase Decontamination Solution (Thermo Fisher Scientific). These can be sprayed on benchtops and pipettes.
- Filtered pipette tips should be used to reduce RNase contamination from pipettes.

Cell Detachment

- ·If using adherent cell line samples, we recommend TrypLE Express Enzyme (1X), phenol red (Thermo Fisher Scientific). Due to high RNase activity, we do not recommend dissociation with standard trypsin, which may reduce gene and transcript detection.

Rigid Plate Strainers

- An example video of using a Rigid Plate Strainer can be found in our Support Suite. We recommend watching this video and practicing using the Rigid Plate Strainer before processing your samples.
- Before using the Rigid Plate Strainer, remember to remove the plastic cover under the strainer.

Cell/Nuclei Counting and Quality Assessment

- We recommend a hemocytometer for counting, but alternative counting devices can also be used. If possible, validate counts from alternative devices to a hemocytometer when first using Evercode Fixation v4 kits.
- When first using Evercode Fixation v4 kits, we suggest saving images at each counting step.
- To assess sample quality, we recommend using viability stains like trypan blue or acridine orange and propidium iodide (AO/PI).
- After fixation, the cells/nuclei are permeabilized and should appear dead with viability stains. If using Acridine Orange/Propidium Iodide (AO/PI) stains, we suggest using the red (PI) channel to count to avoid the impact of any autofluorescence in the green (AO) channel.

- Examples of trypan blue stained fixed cells/nuclei are shown below. High quality fixed samples have single distinct cells/nuclei with <5% cell/nuclei aggregation and no debris. Higher levels of aggregation will lead to elevated doublets after sequencing. When quantifying fixed cells/nuclei, it is critical to avoid counting cell/nuclei debris to avoid overestimating the number of cells/nuclei.

High Quality Sample | Aggregation | Debris

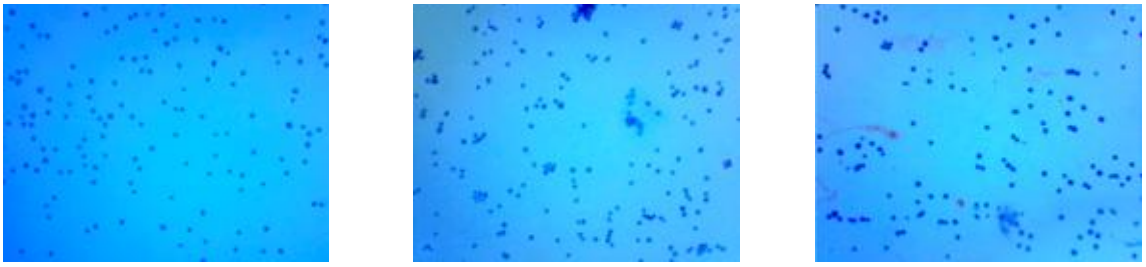


Figure 3: Example of trypan blue stained fixed cells.

Centrifugation

- A range of centrifugation speeds and durations are given in this protocol rather than a single speed. When using Evercode Fixation v4 kits for the first time or when testing a new sample type, we recommend optimizing centrifugation conditions in 1.5 mL tubes before using the plate-based workflows. See the tube-based protocol in our support site, which includes detailed optimization recommendations.

- A swinging bucket rotor should be used for all high-speed centrifugation steps in this protocol. The use of a fixed-angle rotor will lead to substantial cell/nuclei loss.

Reagent Stability

- Reagents in the Cell/Nuclei Fixation Reagents box should not be frozen and thawed more than 3 times.

- If the kit is going to be used more than 3 times, the reagents should be aliquoted into nuclease-free 1.5 mL tubes and stored at -20°C until use. We do not recommend making single use aliquots to minimize the impact of evaporation during storage.

- Reagent master mixes should be made fresh and used the same day.

Storage of Fixed Samples

- Fixed samples can be stored at -80°C for up to 6 months. Fixed samples should not be refrozen after thawing.

- When possible, we recommend splitting samples into aliquots after fixation. Aliquots should be at least 20 µL when stored in 0.2 mL PCR tubes or 96-well plates.

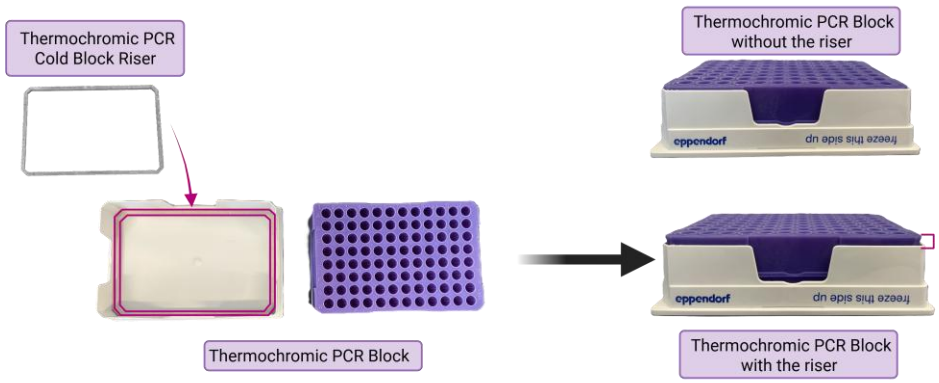
- Counting aliquots can be thawed and counted separately from the remaining sample for downstream processing with Evercode kits. See this Support Suite Article for more information.

Thermochromic PCR Cold Block

- The Thermochromic PCR Cold Block changes color from dark purple to bright pink (too warm) when the block temperature exceeds 7°C. If greater than 30% of the block is bright pink, replace the Thermochromic PCR Cold Block with a fully frozen dark purple block.

- To maintain optimal performance and minimize temperature fluctuations, store the Thermochromic PCR Cold Blocks in a -20°C freezer when not in use. The blocks should be stored upside down to prevent warping.

- The Thermochromic PCR Cold Block Riser needs to be installed in the Thermochromic PCR Cold Block prior to use as illustrated below. The Riser does not need to be removed when freezing.



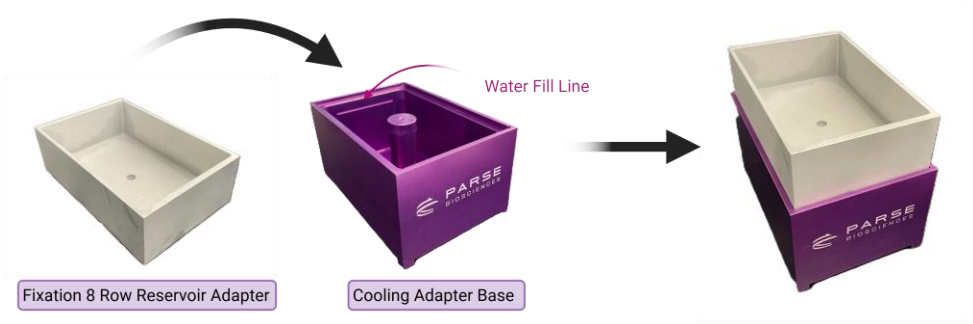
Note: Uneven freezing of the liquid inside the Thermochromic PCR Block can cause slanting of the cooling position. The Thermochromic PCR Cold Block Riser reduces the variability caused by the freezing process. If the PCR Cold Block riser is not used, there will be extra liquid left in the wells of the PCR plate during aspiration steps.

- Tip pinching may occur when using a fully frozen Thermochromic PCR Cold Block. To minimize tip pinching, a fully frozen Thermochromic PCR Cold Block should be warmed slightly by a few degrees by leaving it at room temperature for 10 minutes before using it on the INTEGRA ASSIST PLUS Deck.

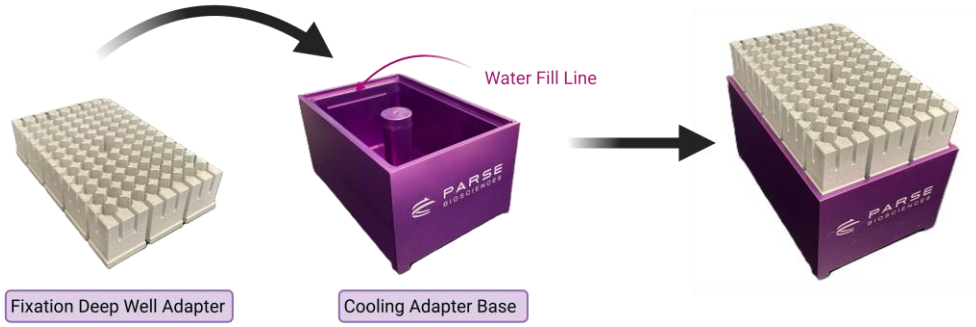
- To ensure a secure positioning within the Thermochromic PCR Cold Block, apply firm pressure on the PCR plates until they are securely seated. Avoid touching the top of the open wells.

Cooling Adapter Bases

- To assemble the Cooling Adapter Bases place the Fixation 8 Row Reservoir Adapter or the Fixation Deep Well Adapter on top of the Cooling Adapter Base as shown in the figures below.



Fixation 8 Row Reservoir Adapter with Cooling Adapter Base.



Fixation Deep Well Adapter with Cooling Adapter Base.

Note: If the Cooling Adapter Base is filled with water and frozen before use, ensure that the water level does not exceed the water fill line. The water will expand when frozen and the adapter will not fit properly if the base is overfilled.

- Before use, the Cooling Adapter Base should be filled with water and frozen the night before, then thawed at room temperature for at least 10 minutes prior to use. Alternatively, it can be filled with pebble ice immediately before being placed on the Integra Deck.

INTEGRA ASSIST PLUS Pipetting Programs

- Ensure that the VIALAB program is installed on the same computer that will be used to communicate with the Pipetting Module.
- Ensure that Evercode workflow script precheck has been confirmed prior to running the INTEGRA + Parse workflow. This can be done by running the Evercode WT with INTEGRA ASSIST PLUS Precheck Scripts available on the Customer Support Suite.
- Automated pipetting programs are set up and can be selected using the pipette's user interface. In the INTEGRA + Parse workflow, prompts are built into the programs as checkpoints. When the prompt is followed by a double dash, "--", the program will continue. If the prompt does not contain a double dash, "--", it is followed by another prompt.

Deck Loading

- To prevent malfunctions during the procedure, ensure that all unnecessary labware is cleared from the deck before initiating a new program on the INTEGRA ASSIST PLUS.

Cell Fixation Reagents

The Evercode Cell Fixation v4, 96 reactions workflow requires Cell Fixation Reagents and Cell Fixation Enhancer boxes. This kit is used for the 48 reactions script as well.

Cell Fixation Reagents, 96 reactions. Store at -20°C, PN CF500

LABEL	ITEM	PN	FORMAT	QTY
	Prefixation Buffer	CF301	15 mL bottle	2
	Storage Buffer	CF302	8 mL bottle	2
	Fixative Solution A	CF303	8 mL bottle	2
	Fixative Solution B	CF304	8 mL bottle	2
	Permeabilization Solution	CF318	2 mL tube	2
	Fix and Perm Stop Buffer	CF306	15 mL bottle	2
	RNase Inhibitor	CF307	1.5 mL tube	1
	DMSO	CF308	1.5 mL tube	1

Cell Prefixation Enhancer, 96 reactions. Store at 4°C, PN CF600

LABEL	ITEM	PN	FORMAT	QTY
	Prefixation Enhancer	CF401	2 mL tube	1

30 µM Rigid Plate Strainer*. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Rigid Plate Strainer, 30 µM	RPS1030	6-pack	1

70 µM Rigid Plate Strainer*. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Rigid Plate Strainer, 70 µM	RPS1070	6-pack	1

100 µM Rigid Plate Strainer*. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Rigid Plate Strainer, 100 µM	RPS1100	6-pack	1

Note: * Only one mesh size of Rigid Plate Strainer is required for the Evercode Cell Fixation v4 workflow. Select an appropriate mesh size for each sample type.

Nuclei Fixation Reagents

The Evercode Nuclei Fixation v4, 96 reactions workflow requires Nuclei Fixation Reagents and Nuclei Fixation Enhancer boxes. This kit is used for the 48 reactions script as well.

Nuclei Fixation Reagents, 96 reactions. Store at -20°C, NF700

LABEL	ITEM	PN	FORMAT	QTY
	Prefixation Buffer	NF301	15 mL bottle	2
	Storage Buffer	NF302	8 mL bottle	2
	Fixative Solution	NF303	8 mL bottle	2
	Permeabilization Solution	NF313	2 mL tube	2
	Fix and Perm Stop Buffer	NF305	15 mL bottle	2
	RNase Inhibitor	NF306	1.5 mL tube	1
	DMSO	NF307	1.5 mL tube	1

Nuclei Fixation Enhancer, 96 reactions. Store at 4°C, NF800

LABEL	ITEM	PN	FORMAT	QTY
	Prefixation Enhancer	NF401	2 mL tube	1

30 µm Rigid Plate Strainer. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Rigid Plate Strainer, 30 µM	RPS1030	6-pack	1

Parse Equipment

The following is a list of equipment provided by Parse Biosciences, required to successfully perform the Evercode workflows on the INTEGRA ASSIST PLUS.

ITEM	PN	QTY	CHECKLIST
Thermochromic PCR Cold Block	NTAC1102	2	☐
Thermochromic PCR Cold Block Riser	NTAC1103	2	☐
Fixation Deep Well Adapter	NTAC1104	2	☐
Fixation 8 Row Reservoir Adapter	NTAC1105	1	☐
Fixation Cooling Adapter Base	NTAC1106	3	☐

INTEGRA Components

The following are required INTEGRA ASSIST PLUS components needed to run the Evercode Fixation workflow on the INTEGRA ASSIST PLUS.

ITEM	ITEM TYPE	PN	QTY	CHECK LIST
Pipette Communication Module for VIAFLO / VOYAGER Pipettes	Accessory	4221	1	☐
ASSIST PLUS Base Unit	Main	4505	1	☐
Communication/Charging Cable for VIAFLO	Accessory	4226	1	☐
VIAFLO Pipette 12-Channel, 10-300 µL	Pipette	4633	1	☐

INTEGRA Consumables

The following is a list of consumables available from INTEGRA, required to successfully perform Evercode Fixation workflow on the INTEGRA ASSIST PLUS.

ITEM	ITEM TYPE	PN	QTY	CHECK LIST
8 Row Reagent Reservoirs, Partitioned (32 mL/row) with SUREFLO design	INTEGRA	6373	4	☐
10-300 µL pipette tips (Sterile/Filter/Low Retention)	INTEGRA	6535	6 (48RXN) or 9 (96 RXN)	☐

Consumables

The following equipment and consumables are required to perform the protocol, but are not provided by INTEGRA or by Parse Biosciences. Note that this list does not include standard laboratory equipment, such as freezers.

Consumables

ITEM	SUPPLIER	PN	NOTES	CHECK LIST
SealPlate®	Excel Scientific®	100-SEAL-PLT	Or equivalent PCR plate seals. Note that many clear plastic seals are not designed for storage at -80°C, so we recommend using foil plate seals if storing fixed samples in PCR plates.	☐
TempPlate® EXT Sealing Foil	USA Scientific®	2998-0100	If storing fixed samples in a PCR plate. Note that many clear plastic seals are not designed for storage at -80°C.	☐
Protein LoBind® Plate	Eppendorf®	951033308 (1 mL)	Or equivalent polypropylene, nuclease-free, v-bottom, 1 mL deep well plates. Do not substitute polystyrene plates as it will lead to substantial cell loss. If possible, we recommend using protein low-binding plates.	☐
Rigid Plate Strainer	Parse Biosciences	RPS1030 RPS1070 RPS1100	Choose an appropriate mesh size for your sample type.	
INTEGRA 25 mL polypropylene reservoirs	INTEGRA	4317	(Optional) If blocking deep well plates with BSA. Or any sterile, nuclease-free reagent basins.	☐
Eppendorf twin.tec® PCR Plate 96 LoBind®	Eppendorf	0030129504	Or equivalent DNA low-binding, nuclease-free PCR plate or 0.2 mL tube strips.	☐
Falcon® High Clarity PP Centrifuge Tubes, 15 mL	Corning®	352097 (15 mL)	Or equivalent polypropylene centrifuge tubes.	☐

ITEM	SUPPLIER	PN	NOTES	CHECK LIST
Pipette Tips RT LTS 20 µL FL 960A/10 RT LTS 200 µL FL 960A/10 RT LTS 1000 µL FL 768A/8	Rainin®	30389226 30389240 30389213	Or appropriate DNA low-binding, DNase/RNase-free, and filtered pipette tips. Do not use wide bore tips.	☐

Equipment

ITEM	SUPPLIER	PN	NOTES	CHECK LIST
Centrifuge with Swinging Bucket Rotor	Various Suppliers	Varies	Compatible with 96 deep well plates and capable of reaching 4°C.	☐
Microcentrifuge	Various Suppliers	Varies	Compatible with 1.5 mL tubes.	☐
Single-channel pipette: P20, P200, P1000	Various Suppliers	Varies		☐
Hemocytometer	Sigma-Aldrich®	Z359629	Or other cell counting devices. We recommend validating alternatives relative to a hemocytometer.	☐
Polystyrene foam cooler	Various Suppliers	Varies	(Optional) If storing fixed samples before processing with an Evercode Whole Transcriptome kit.	☐
Plate Seal Applicator	Various Suppliers	Varies	Capable of adhering plate sealing films to 96 well plates.	☐

Reagents

ITEM	SUPPLIER	PN	NOTES	CHECK LIST
Fluorescence dyye	Various Suppliers	Varies	Any dye to assess cell viability, such as AO/PI.	☐
RNaseZap™ RNase Decontamination Solution	Thermo Fisher Scientific	AM9780	Or equivalent RNase decontamination solution.	☐
Gibco™ Bovine Albumin Fraction V (7.5% solution)	Thermo Fisher Scientific	15260037	(Optional) If blocking tubes with BSA. Chosen due to its low RNase activity. Contact applications support for alternatives.	☐

Reading and Understanding Visuals and Diagrams

Understanding the figures in this user manual is essential for accurately interpreting the information presented.

While the figures are different, they share consistent formats, use standardized visual elements and follow common conventions.

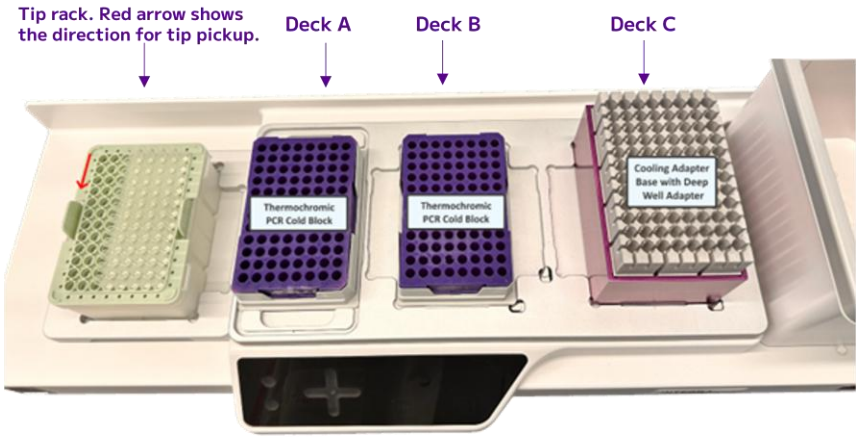
There are three types of figures in this manual.

- Photos of the hardware configurations, including tip rack, and Decks A, B, and C.
- Visual representation of Decks A, B, and C where consumable labware, reagents, and samples are placed.
- Diagrams of the pipette illustrating the steps to select the appropriate program.

We recommend reviewing this section and the illustrations throughout the manual to familiarize yourself with the visual language used.

Understanding the Hardware Configurations

The hardware configurations appear at the beginning of each subsection and show how to set up the INTEGRA platform to complete the associated steps. This photo displays the tip racks, Deck A, Deck B, and Deck C. Red arrows indicate the order in which tips are picked up by the INTEGRA platform, and required hardware is highlighted with text boxes.

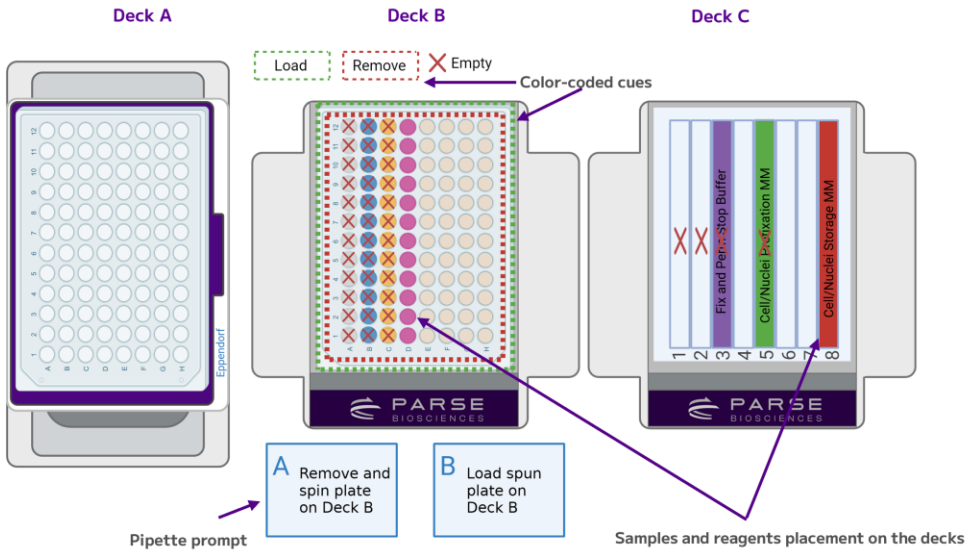


Blocks and Bases are hardware components that will be placed or removed as needed throughout the workflow.

Reading and Understanding the Deck Configurations

Each subsection includes multiple deck configurations that illustrate the steps executed by the program. These configurations display the exact placement of reagents and samples on the

decks, list the consumables required at each step, and highlight pipette prompts for manual intervention. Color-coded cues guide the user through actions such as loading, moving, or removing hardware, reagents, and consumables, while also indicating the specific deck locations where these actions should occur.



Color-Coded Cues

This workflow is semi-automated and still requires user interaction and input. Prompts will appear on the pipette display, instructing the user to perform tasks such as loading, moving, removing, or replacing hardware, reagents, and consumables, as well as mixing and pipetting specific reagents. In this user guide, each prompt is color-coded and shown in the deck configurations illustrating the required actions. Additional explanatory text accompanies each figure to provide further detail on the steps involved.

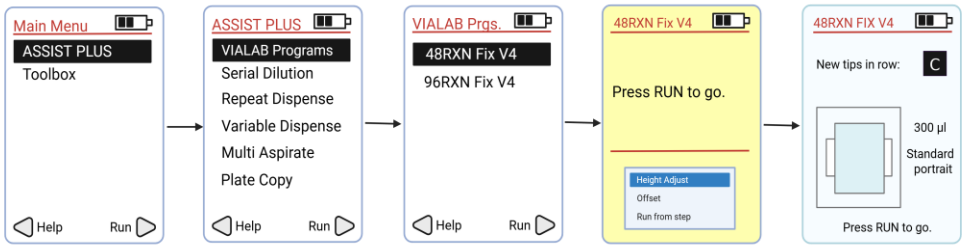
- **Load**: Indicates labware and/or reagents that need be placed or reloaded onto the deck.
- **Remove**: Identifies labware that should be taken off the deck, either for incubation, disposal, or because it is not needed in the upcoming step.
- **Replace**: Highlights labware that has been used and needs to be swapped out for clean or new consumables.
- **Move**: Indicates labware that should be relocated between deck positions.

- **X Emptied**: Reminds that the content has been used and the vessel is now empty.

Note: The position of the color-coded cues on top of the figures does not necessarily correspond to the location where the action should be taken.

Pipette Programs Diagram

At various points in the procedure, the user will need to switch pipettes and tip racks. In each subsection, the pipette is pre-programmed to carry out specific tasks defined by a designated script. The correct program must be loaded onto the pipette before beginning each set of steps. A diagram showing the path to load the appropriate program is provided in every subsection.



Section 1: Set Up

Section 1.1. Block Plates with BSA

Although not required, blocking plates with BSA can increase cell retention. When Protein LoBind plates are not available, we recommend blocking plates, especially for samples with low cell inputs or cells prone to aggregation.

To block plates:

1. Prepare a fresh 1% BSA as follows, depending on the number of samples being processed.

1% BSA		
Number of Samples	48	96
Nuclease-free water (not supplied)	87 mL	175 mL
Gibco Bovine Albumin Fraction V (7.5% solution) (not supplied)	13 mL	27 mL
Total Volume	100 mL	202 mL

2. Fill a new basin with 1% BSA, refilling as needed in step 3.
3. For each sample, add **1 mL** of 1% BSA to a well of a polypropylene, nuclease-free, v-bottom, 1 mL Deepwell Plate.
4. Repeat step 3 with a second Deepwell Plate.
5. Add new plate seals and invert once to fully coat the wells.
6. Incubate for **30 minutes** at room temperature.
7. Remove the plate seals. Decant and discard the 1% BSA.
8. Add new plate seals.
9. Centrifuge the plates for **1 minute** at 100 x g at room temperature.
10. Remove the plates from the centrifuge and remove the plate seals.
11. Remove any remaining solution from the bottom of the wells with a multichannel P200.

12. Without sealing the plates, air dry for **30 minutes** in a biosafety cabinet at room temperature.

13. Proceed to Section 1.2 or store sealed BSA-coated plates at 4°C for up to 4 weeks.

Section 1.2. Prepare Master Mixes for Cell Fixation

Cell fixation master mixes should be prepared just prior to fixation. The tables in this section show volumes for 48- and 96-sample runs; volumes dispensed will vary by script.

1. Fill a bucket with ice. Gather the following items and handle as indicated below.

ITEM	SOURCE	QTY	HANDLING AND STORAGE
8 Row Reservoir Insert	INTEGRA-Provided	1	Individually wrapped consumable
1 mL 96 Deepwell plate	Consumables	1	
Fixation Cooling Adapter Base	Parse-Provided	1	If previously frozen, leave at room temperature for 10 minutes prior to use. If not previously frozen, fill with pebble ice prior to use. Replace ice as necessary.
Fixation 8 Row Reservoir Adapter	Parse-Provided	1	
○ Prefixation Buffer	Cell Fixation Reagents (-20°C)	15 mL bottle	Thaw at room temperature then immediately store on ice. Mix by inverting each tube/bottle. Do not vortex.
○ Storage Buffer	Cell Fixation Reagents (-20°C)	8 mL bottle	
○ Fixative Solution A	Cell Fixation Reagents (-20°C)	8 mL bottle	
○ Fixative Solution B	Cell Fixation Reagents (-20°C)	8 mL bottle	
● Permeabilization Solution	Cell Fixation Reagents (-20°C)	2 mL tube	
○ Fix and Perm Stop Buffer	Cell Fixation Reagents (-20°C)	15 mL bottle	
● DMSO	Cell Fixation Reagents (-20°C)	1.5 mL tube	Thaw and store at room temperature. Mix by inverting the tube.
● RNase Inhibitor	Cell Fixation Reagents (-20°C)	1.5 mL tube	Store on ice immediately before use. Do not vortex.

ITEM	SOURCE	QTY	HANDLING AND STORAGE
● Prefixation Enhancer	Cell Fixation Reagents (4°C)	2 mL tube	

2. Prepare the Cell Prefixation Master Mix in a new tube as follows. Mix thoroughly by pipetting and pipette into **row 5** of the 8 Row Reservoir. Store the 8 Row Reservoir on ice.

CELL PREFIXATION MASTERMIX		
Number of Samples	48	96
○ Prefixation Buffer	10.7 mL	21.3 mL
● RNase Inhibitor	144 µL	288 µL
● Prefixation Enhancer	720 µL	1.4 mL
Total Volume	11.5 mL	23 mL

3. Prepare the Cell Fixative Master Mix in a new tube as follows. Mix thoroughly by pipetting and pipette into **row 6** of the 8 Row Reservoir. Store the 8 Row Reservoir on ice.

CELL FIXATIVE MASTERMIX		
Number of Samples	48	96
○ Fixative Solution A	2.4 mL	4.8 mL
○ Fixative Solution B	2.4 mL	4.8 mL
Total Volume	4.8 mL	9.6 mL

4. Prepare the Cell Storage Master Mix in a new tube as follows. Mix thoroughly by pipetting and pipette into **row 8** of the 8 Row Reservoir. Store the 8 Row Reservoir on ice.

CELL STORAGE MASTERMIX		
Number of Samples	48	96
O Storage Buffer	6.3 mL	12.6 mL
● RNase Inhibitor	84 µL	168 µL
● DMSO	336 µL	672 µL
Total Volume	6.72 mL	13.44 mL

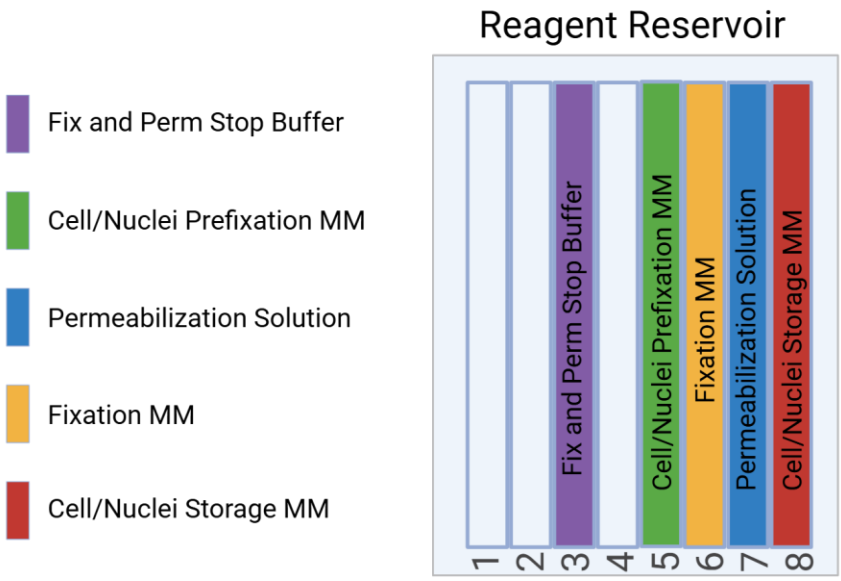
5. Dispense all of the ● Permeabilization Solution into **row 7** of the 8 Row Reservoir. Store the 8 Row Reservoir on ice.

PERMEABILIZATION SOLUTION		
Number of Samples	48	96
● Permeabilization Solution	~2 mL	~4 mL

6. Dispense all of the O Fix and Perm Stop Buffer into **row 3** of the 8 Row Reservoir. Store the 8 Row Reservoir on ice.

FIX AND PERM STOP BUFFER		
Number of Samples	48	96
O Fix and Perm Stop Buffer	~14 mL	~28 mL

7. The 8-Row Reservoir should correspond to the figure below.



CRITICAL! Ensure that the liquid is distributed across the bottom of the reservoir.

Section 1.3. Prepare Master Mixes for Nuclei Fixation

Nuclei fixation master mixes should be prepared just prior to fixation. The tables in this section show volumes for 48- and 96-sample runs; volumes dispensed will vary by script.

1. Fill a bucket with ice. Gather the following items and handle as indicated below.

ITEM	SOURCE	QTY	HANDLING AND STORAGE
8 Row Reservoir Insert	INTEGRA-Provided	1	Individually wrapped consumable
1 mL 96 Deepwell plate	Consumables	1	
Fixation Cooling Adapter Base	Parse-Provided	1	If previously frozen, leave at room temperature for 10 minutes prior to use. If not previously frozen, fill with pebble ice prior to use. Replace ice as necessary.
Fixation 8 Row Reservoir Adapter	Parse-Provided	1	
○ Prefixation Buffer	Nuclei Fixation Reagents (-20°C)	15 mL bottle	Thaw at room temperature then immediately store on ice. Mix by inverting each tube/bottle. Do not vortex.
○ Storage Buffer	Nuclei Fixation Reagents (-20°C)	8 mL bottle	
○ Fixative Solution	Nuclei Fixation Reagents (-20°C)	8 mL bottle	
● Permeabilization Solution	Nuclei Fixation Reagents (-20°C)	2 mL tube	
○ Fix and Perm Stop Buffer	Nuclei Fixation Reagents (-20°C)	15 mL bottle	
● DMSO	Nuclei Fixation Reagents (-20°C)	1.5 mL tube	Thaw and store at room temperature. Mix by inverting the tube.
● RNase Inhibitor	Nuclei Fixation Reagents (-20°C)	1.5 mL tube	Store on ice immediately before use. Do not vortex.
● Prefixation Enhancer	Nuclei Fixation Reagents (4°C)	2 mL tube	

2. Prepare the Nuclei Prefixation Master Mix in a new tube as follows. Mix thoroughly by pipetting and pipette into **row 5** of the 8 Row Reservoir. Store the 8 Row Reservoir on ice.

NUCLEI PREFIXATION MASTER MIX		
Number of Samples	48	96
○ Prefixation Buffer	10.7 mL	21.3 mL
● RNase Inhibitor	144 µL	288 µL
● Prefixation Enhancer	720 µL	1.4 mL
Total Volume	11.5 mL	23 mL

3. Dispense all of the ○ Fixative Solution Nuclei Fixative Solution into **row 6** of the 8 Row Reservoir. Store the 8 Row Reservoir on ice.

NUCLEI FIXATIVE SOLUTION		
Number of Samples	48	96
○ Fixative Solution	~5.2 mL	~10.4 mL

4. Prepare the Nuclei Storage Master Mix in a new tube as follows. Mix thoroughly by pipetting and pipette into **row 8** of the 8 Row Reservoir. Store the 8 Row Reservoir on ice.

NUCLEI STORAGE MASTER MIX		
Number of Samples	48	96
○ Storage Buffer	6.3 mL	12.6 mL
● RNase Inhibitor	84 µL	168 µL
● DMSO	336 µL	672 mL
Total Volume	6.72 mL	13.44 mL

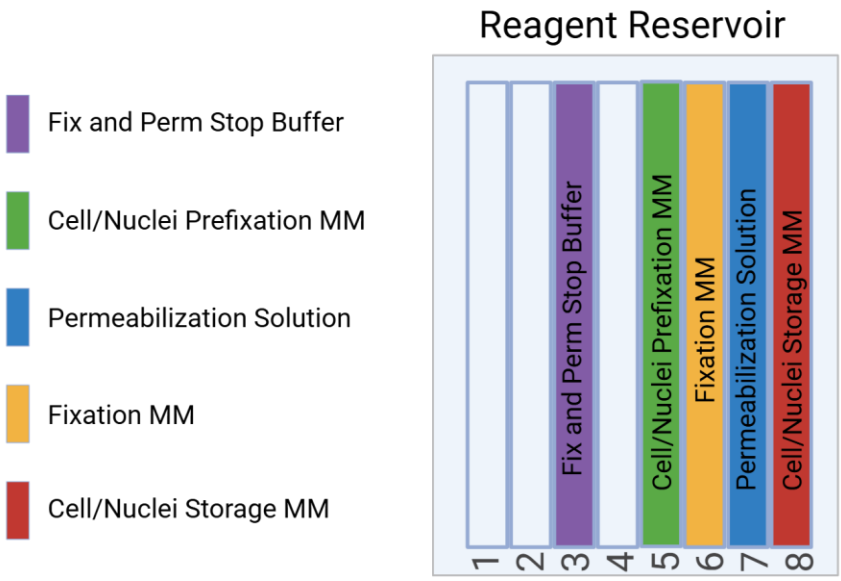
5. Dispense all of the ● Permeabilization Solution into **row 7** of the 8 Row Reservoir. Store the 8 Row Reservoir on ice.

PERMEABILIZATION SOLUTION		
Number of Samples	48	96
● Permeabilization Solution	~2 mL	~4 mL

6. Dispense all of the ○ Fix and Perm Stop Buffer into **row 3** of the 8 Row Reservoir. Store the 8 Row Reservoir on ice.

FIX AND PERM STOP BUFFER		
Number of Samples	48	96
○ Fix and Perm Stop Buffer	~14 mL	~28 mL

7. The 8-Row Reservoir should correspond to the figure below.



CRITICAL! Ensure that the liquid is distributed across the bottom of the reservoir.

Section 2: Fixation

Section 2.1. 48/96 Reactions Fixation

This section outlines the protocol for processing 48 and/or 96 samples. When processing 48 samples the procedure takes approximately 45 minutes to complete, with an additional 10 minutes required for centrifugation. If processing 96 samples the procedure requires about 80 minutes plus 10 minutes for centrifugation.

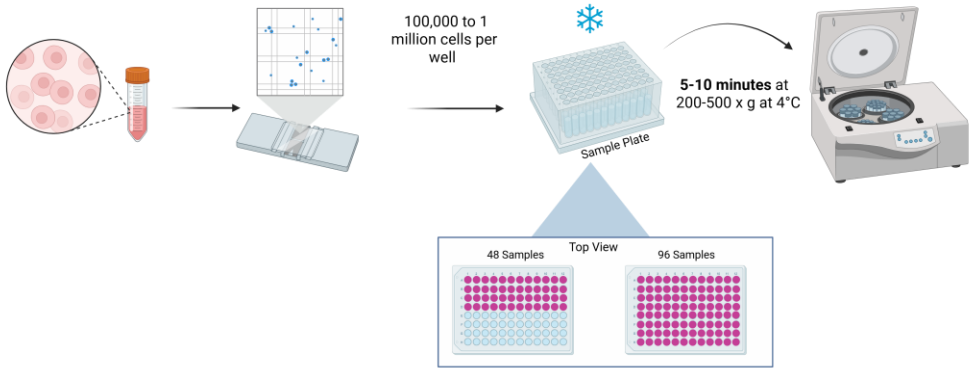
To fix samples:

1. Gather the following components:

ITEM	SOURCE	QTY	HANDLING AND STORAGE
VIAFLO Pipette 12-Ch, 10-300 µL	INTEGRA Component	1	
Tip Deck for VIAFLO Pipetting Module	INTEGRA Component	1	
10-300 µL Tip Rack	INTEGRA-Provided	1	
Thermochromic PCR Cold Block	Parse-Provided	2	Pull the Freezer Block with stabilizer from the -20°C freezer and leave them at room temperature for 10 minutes prior to use.
Thermochromic PCR Cold Block Riser	Parse-Provided	2	
Fixation Deep Well Adapter	Parse-Provided	2	
Fixation 8 Row Reservoir Adapter	Parse-Provided	1	
Fixation Cooling Adapter Base	Parse-Provided	3	If previously frozen, leave at room temperature for 10 minutes prior to use. If not previously frozen, fill with pebble ice prior to use. Replace ice as necessary.
SealPlate®	Consumables	as needed	
TempPlate® EXT Sealing Foil	Consumables	as needed	

ITEM	SOURCE	QTY	HANDLING AND STORAGE
Rigid Plate Strainer	Parse-Provided	2	Choose an appropriate mesh size for your sample type
Protein LoBind® Plate	Consumables	2	
Eppendorf twin.tec® PCR Plate 96 LoBind®	Consumables	2	

2. Cool the centrifuge with a swinging bucket rotor to 4°C.
3. Fill a bucket with ice.
4. Prepare sample input:



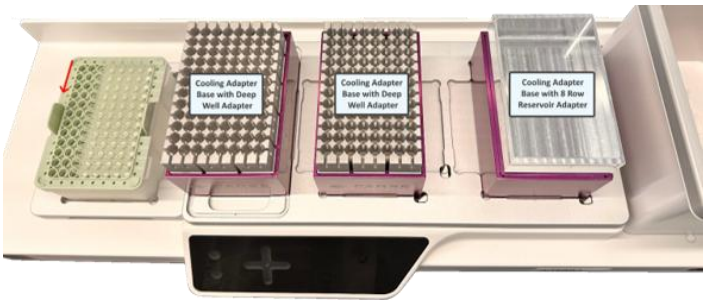
   a. Prepare a hemocytometer, or other cell/nuclei counting device.
   b. Count the cells/nuclei in the single cell suspension with a hemocytometer or alternative counting device and record the count. Keep cells/nuclei on ice during counting and work quickly to minimize time on ice prior to fixation.
   c. With the plate on ice, transfer 100,000 to 1 million cells/nuclei from each sample into the wells of a 1 mL deep well plate (or BSA-coated deep well plate if prepared in Section 1.1). Maximum sample volume per well is 1 mL.
      i. Row A - Row D for 48 Samples
      ii. Row A - Row H for 96 Samples
   d. Place a new plate seal on the deep well plate containing the cell suspension.

e. Centrifuge the plate in a swinging bucket rotor for **5-10 minutes** at 200-500 x g at 4°C. Proceed to Integra deck preparation during centrifugation.

CRITICAL! Using a fixed-angle rotor in this protocol will lead to substantial cell loss.

CRITICAL! Ideal centrifugation speed and duration should be determined for each sample type to optimize retention and resuspension efficiencies. See the Important Guidelines section for details.

5. Prepare the cooling hardware:



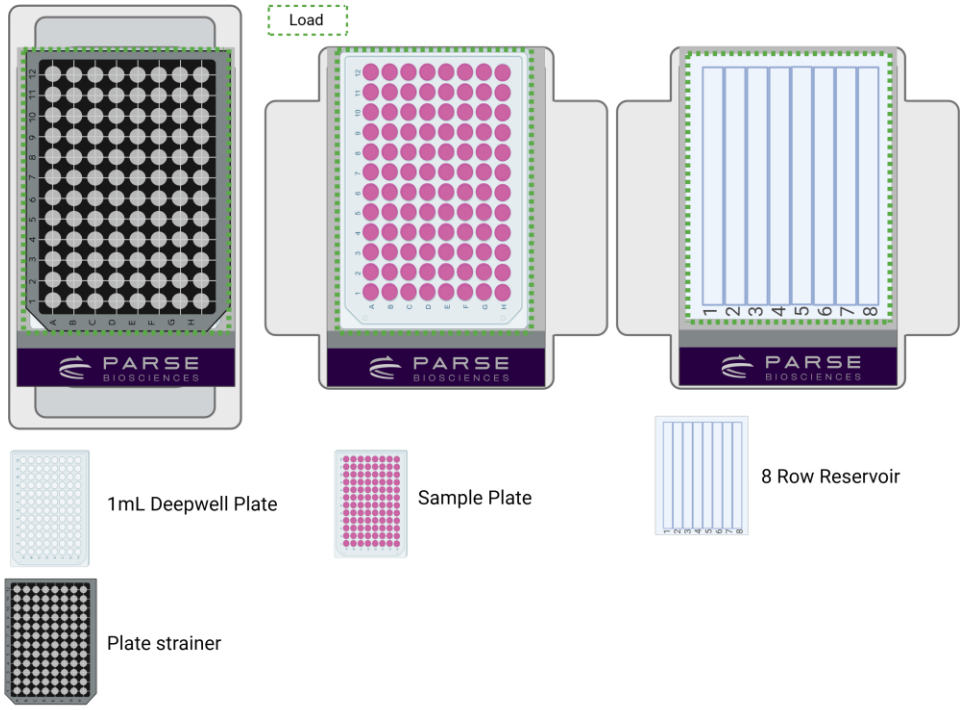
a. Assemble and fill three of the Cooling Adapter Bases with pebble ice and place one on Deck A, Deck B, and Deck C. If the Cooling Adapter Bases were filled with water and frozen the night before, ensure that the Cooling Adapter Base is thawed at room temperature for at least **10 minutes** before use.

CRITICAL! If using the pebble ice filled Cooling Adapter Bases for more than 45 minutes, refill the cooling base with more pebble ice at the 40 minute mark. If the cooling adapters were frozen overnight, refill them with pebble ice at the 90 minute mark.

b. Place the Fixation Deep Well Adapter on the Cooling Adapter Bases on Deck A and Deck B.

c. Place the Fixation 8 Row Reservoir Adapter on the Cooling Adapter Base on Deck C.

6. Set up the deck following the Deck Configuration below.



   a. Place a **new** 8 Row Reservoir on the Fixation 8 Row Reservoir Adapter on Deck C. This will be the sample waste reservoir.

   b. Put a Rigid Plate Strainer on a **clean** 1 mL deep well plate and place it on top of the Fixation Deep Well Adapter on Deck A with A1 on the bottom left corner. Remove the clear film on the bottom of the strainer prior to loading on deck. Press firmly to ensure proper straining.

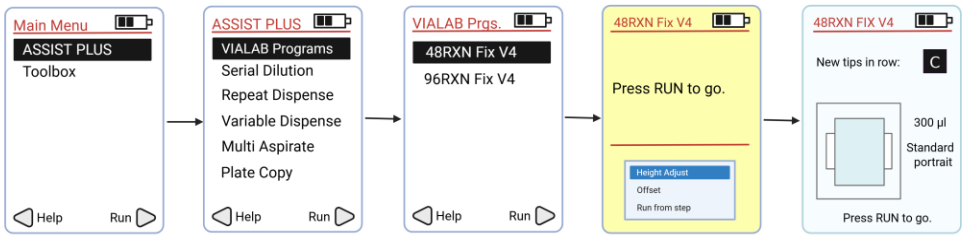
Note: Make sure to choose an appropriate mesh size for the sample type.

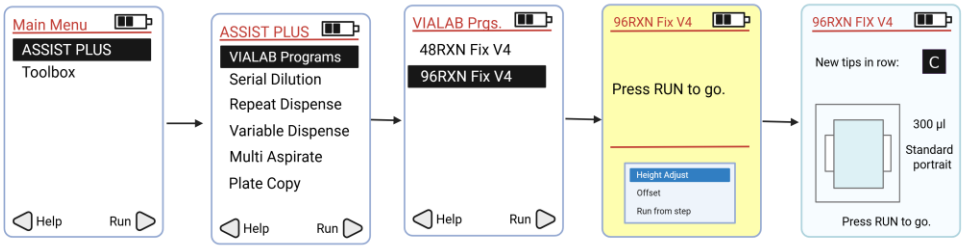
   c. Once the centrifugation is complete, place the 1 mL deep well plate containing the samples on top of the Fixation Deep Well Adapter on Deck B, and remove the plate seal.

CRITICAL! Move quickly and handle the sample gently to avoid dislodging the pellet, which will impact data quality.

7. Attach VIAFLO Pipetting Module 12-Ch, 10-300 µL and the corresponding Tip Deck. Ensure the Tip Deck and tip box are securely positioned by pressing them down firmly until they click into place. Remove the tip box lid prior to starting the program.

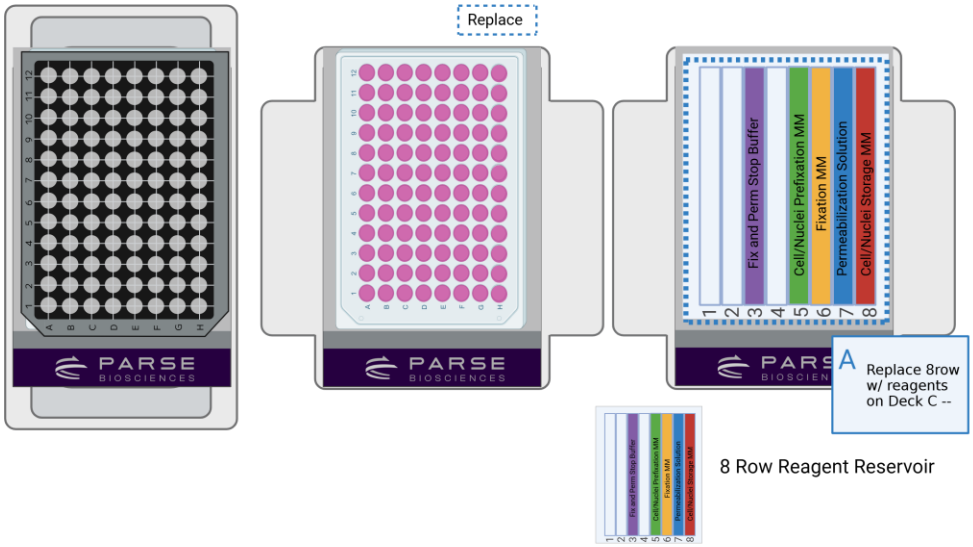
8. Run the program **48RXN Fix V4** if running up to 48 samples, or program **96RXN Fix V4** if running up to 96 samples.



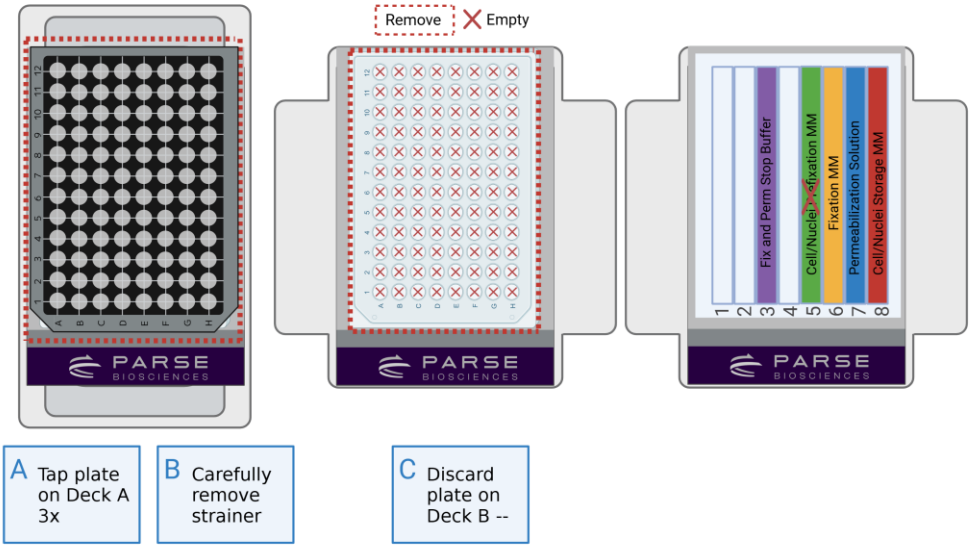


Note: During the supernatant removal from the 1 mL deep well plate containing the samples, the pipette tip should not touch the sides of the 8 row reservoir. The tip movement is intended to remove large droplets on the outside of the pipette tip.

9. **When prompted**, replace the cell waste reservoir on Deck C with the Reagent 8 Row Reservoir prepared in Section 1. The deck layout should correspond to the configuration below.



10. Press "Run" to continue. Follow the program prompts for manual intervention:



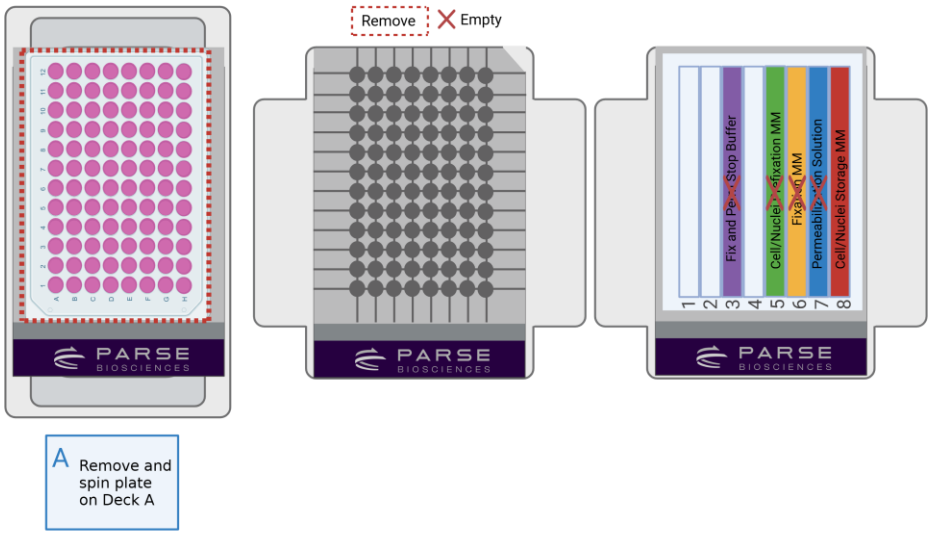
a. Tap the plate on Deck A firmly 3x on the deck to move liquid to the bottom of the wells. If there is a straining failure, manual recovery is recommended. Use a 200 µL multichannel pipette set to 150 µL, aspirate the unstrained liquid pooled on the strainer, press firmly on the strainer prior to dispensing.

b. Carefully remove the strainer on the 96 deep well plate on Deck A and discard.

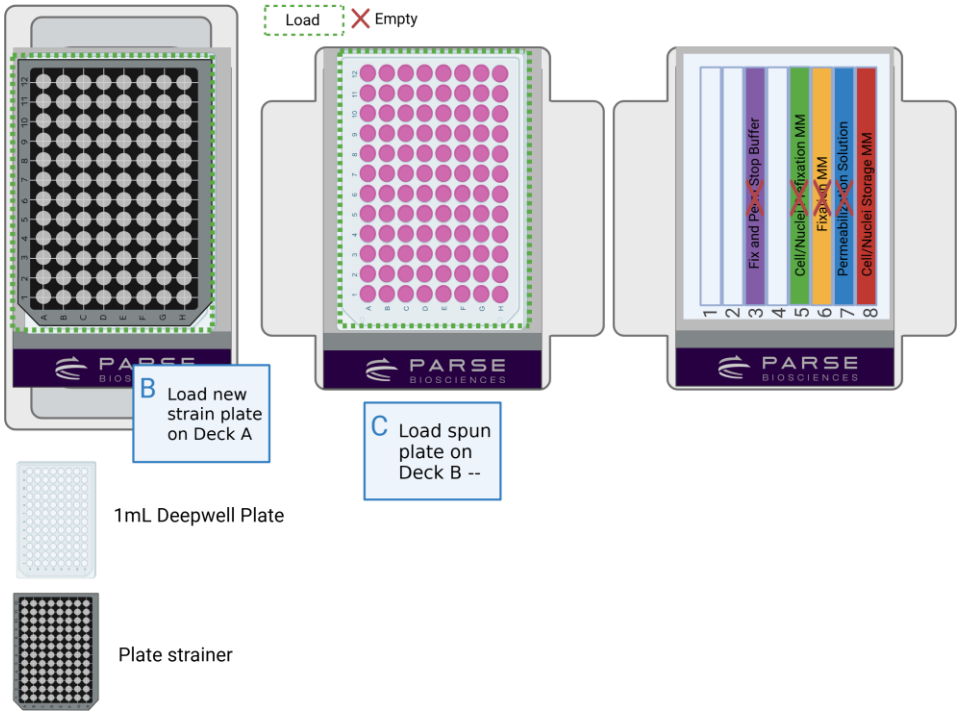
CRITICAL! Remove the strainer by lifting straight up to reduce the chances of contamination.

c. Discard the 96 deep well plate on Deck B.

11. Press "Run" to continue the program. Follow the program prompts for manual intervention:



a. Seal and centrifuge the plate on Deck A in a swinging bucket rotor for **5-10 minutes** at 200-500 x g at 4°C. Continue to the next step during centrifugation.



b. Apply a new Rigid Plate Strainer to a new 1 mL 96 deep well plate by peeling off the backing, carefully aligning over the wells A1-A12, and placing on the surface

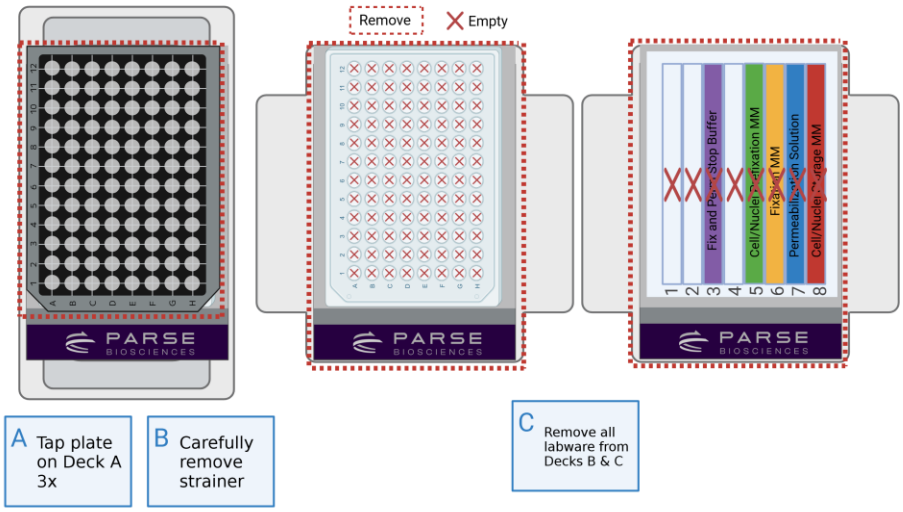
of the plate. Place this plate on the Deep Well Plate Cooling Adapter on Deck A with A1 towards the bottom left. Press firmly to ensure proper straining.

CRITICAL! Ensure your plate strainer is properly aligned to the top of your wells.

CRITICAL! Move quickly and handle the sample gently to avoid dislodging the pellet, which will impact data quality.

c. Once the centrifugation is complete, place the 1 mL deep well plate containing the sample on top of the Eppendorf 1mL Deepwell Plate Cooling Adapter on Deck B and remove the plate seal.

12. Press "Run" to continue the program. Follow the program prompts for manual intervention:



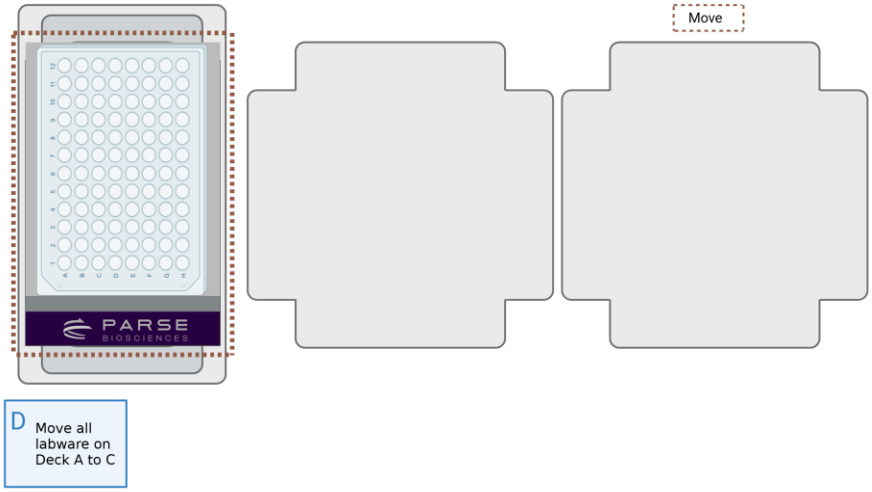
a. Tap the plate on Deck A 3x on the deck to move liquid to the bottom of the wells. If there is a straining failure, manual recovery is recommended. Use a 200 µL multichannel pipette set to 150 µL, aspirate the unstrained liquid pooled on the strainer, press firmly on the strainer prior to dispensing.

b. Carefully remove the strainer and discard.

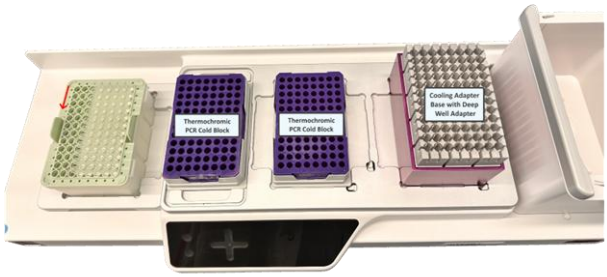
CRITICAL! See guidelines "Storage of Fixed Samples" if you prefer to skip the following steps and abort the program. Proceed directly to step 13.

CRITICAL! Remove the strainer by lifting straight up to reduce the chances of contamination.

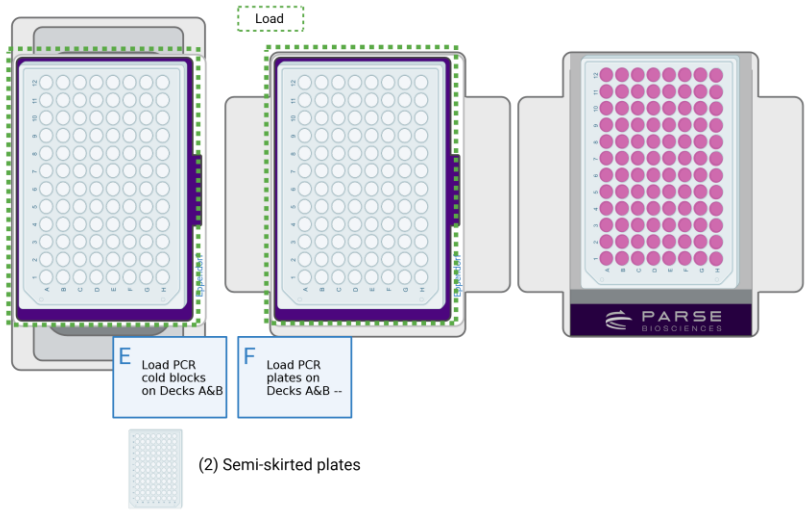
c. Remove all labware on Decks B and C.



d. Move all labware from Deck A to Deck C. This includes the Cooling adapter base with the Deepwell Plate Cooling Adapter and the sample deep well plate.



e. Place 2 Thermochromic PCR Cold Blocks with Risers on both Decks A and B.



f. Place 2 new Eppendorf semi-skirted plates on the Thermochromic PCR Cold Blocks on both Decks A and B.

13. At the end of the program, seal the plates with a foil plate seal, then add a plastic plate seal on top of the foil plate seal. The semi-skirted plate on Deck A contains a 20 µL for each sample in the **Counting Aliquot**. The semi-skirted plate on Deck B contains the fixed samples in the **Sample Plate**.

Note: If proceeding from step 11, and aborted the program before sample transfer into the semi-skirted plates, seal the 1 mL Deep Well plate containing the samples or transfer the samples into a container with a foil seal that can withstand storage at -80°C.

Note: The counting aliquots can be thawed and counted separately from the remaining sample for downstream processing with Evercode kits.

CRITICAL! Many clear plastic seals are not designed for storage at -80°C, so we recommend using foil plate seals is storing fixed samples in PCR plates.

14. Place the samples in a room temperature polystyrene foam cooler, close the lid, and store at -80°C to slowly cool the samples.

Safe stopping point: samples are stable for up to 6 months at -80°C.

15. The day before running the downstream Evercode Whole Transcriptome kit, thaw the 20 µL aliquots in the semi-skirted plate in a water bath or thermocycler set to 37°C. Count the cells in the single cell suspension with a hemocytometer or alternative counting device and record the count. Discard any remaining sample from the counting aliquot.

Appendices

Appendix A: Pipetting Programs

48 _RXN_FIX V4

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Row 1: Remove SN 1	0 min 48 sec
3	Row 1: Remove SN 2	0 min 15 sec
4	Row 1: Remove SN 3	0 min 19 sec
5	Row 2: Remove SN 1	0 min 45 sec
6	Row 2: Remove SN 2	0 min 15 sec
7	Row 2: Remove SN 3	0 min 19 sec
8	Row 3: Remove SN 1	0 min 45 sec
9	Row 3: Remove SN 2	0 min 15 sec
10	Row 3: Remove SN 3	0 min 19 sec
11	Row 4: Remove SN 1	0 min 45 sec
12	Row 4: Remove SN 2	0 min 15 sec
13	Row 4: Remove SN 3	0 min 19 sec
14	Volume Modification	0
15	Row 1: Cell Prefixation Master Mix	1 min 4 sec
16	Row 1: Strain Cells 1	0 min 12 sec
17	Row 1: Remove Excess	0 min 17 sec
18	Row 2: Cell Prefixation Master Mix	1 min 4 sec
19	Row 2: Strain Cells 1	0 min 12 sec
20	Row 2: Remove Excess	0 min 17 sec

STEPS	ACTION	DURATION
21	Row 3: Cell Prefixation Master Mix	1 min 4 sec
22	Row 3: Strain Cells 1	0 min 12 sec
23	Row 3: Remove Excess	0 min 17 sec
24	Row 4: Cell Prefixation Master Mix	1 min 4 sec
25	Row 4: Strain Cells 1	0 min 12 sec
26	Row 4: Remove Excess	0 min 17 sec
27	Prompt to tap plate	0
28	Prompt to remove strainer	0
29	Prompt to discard plate on Deck B	0
30	Add Fixation Solution	2 min 49 sec
31	Fixation Soln Incubation	7 min 0 sec
32	Add Perm Solution	2 min 48 sec
33	Perm Incubation	0 min 30 sec
34	Add Fix and Perm Stop Buffer	3 min 3 sec
35	Row 1: Remove SN 1	0 min 22 sec
36	Row 1: Remove SN 2	0 min 15 sec
37	Row 1: Remove SN 3	0 min 19 sec
38	Row 2: Remove SN 1	0 min 22 sec
39	Row 2: Remove SN 2	0 min 15 sec
40	Row 2: Remove SN 3	0 min 19 sec
41	Row 3: Remove SN 1	0 min 23 sec
42	Row 3: Remove SN 2	0 min 15 sec
43	Row 3: Remove SN 3	0 min 18 sec
44	Row 4: Remove SN 1	0 min 22 sec

STEPS	ACTION	DURATION
45	Row 4: Remove SN 2	0 min 15 sec
46	Row 4: Remove SN 3	0 min 18 sec
47	Row 1: Cell Storage Master Mix	0 min 33 sec
48	Row 1: Resusp 1	0 min 18 sec
49	Row 1: Resusp 2	0 min 18 sec
50	Row 1: Strain Cells 1	0 min 41 sec
51	Row 1: Remove Excess	0 min 17 sec
52	Row 2: Cell Storage Master Mix	0 min 33 sec
53	Row 2: Resusp 1	0 min 18 sec
54	Row 2: Resusp 2	0 min 18 sec
55	Row 2: Strain Cells 1	0 min 41 sec
56	Row 2: Remove Excess	0 min 17 sec
57	Row 3: Cell Storage Master Mix	0 min 33 sec
58	Row 3: Resusp 1	0 min 18 sec
59	Row 3: Resusp 2	0 min 18 sec
60	Row 3: Strain Cells 1	0 min 41 sec
61	Row 3: Remove Excess	0 min 17 sec
62	Row 4: Cell Storage Master Mix	0 min 33 sec
63	Row 4: Resusp 1	0 min 18 sec
64	Row 4: Resusp 2	0 min 18 sec
65	Row 4: Strain Cells 1	0 min 41 sec
66	Row 4: Remove Excess	0 min 17 sec
67	Prompt to tap plate.	0
68	Prompt to remove the cell strainer.	0

STEPS	ACTION	DURATION
69	Prompt to remove labware.	0
70	Prompt to move labware	0
71	Create Counting Aliquot Plate	2 min 24 sec
72	Create Sample Storage Plate	2 min 23 sec

96_RXN_FIX V4

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Row 1: Remove SN 1	0 min 48 sec
3	Row 1: Remove SN 2	0 min 15 sec
4	Row 1: Remove SN 3	0 min 19 sec
5	Row 2: Remove SN 1	0 min 45 sec
6	Row 2: Remove SN 2	0 min 15 sec
7	Row 2: Remove SN 3	0 min 19 sec
8	Row 3: Remove SN 1	0 min 45 sec
9	Row 3: Remove SN 2	0 min 15 sec
10	Row 3: Remove SN 3	0 min 19 sec
11	Row 4: Remove SN 1	0 min 45 sec
12	Row 4: Remove SN 2	0 min 15 sec
13	Row 4: Remove SN 3	0 min 19 sec
14	Row 5: Remove SN 1	0 min 45 sec
15	Row 5: Remove SN 2	0 min 15 sec
16	Row 5: Remove SN 3	0 min 19 sec
17	Row 6: Remove SN 1	0 min 45 sec
18	Row 6: Remove SN 2	0 min 15 sec

STEPS	ACTION	DURATION
19	Row 6: Remove SN 3	0 min 19 sec
20	Row 7: Remove SN 1	0 min 45 sec
21	Row 7: Remove SN 2	0 min 15 sec
22	Row 7: Remove SN 3	0 min 19 sec
23	Row 8: Remove SN 1	0 min 45 sec
24	Row 8: Remove SN 2	0 min 15 sec
25	Row 8: Remove SN 3	0 min 19 sec
26	Volume Modification	0
27	Row 1: Cell Prefixation Master Mix	1 min 3 sec
28	Row 1: Strain Cells 1	0 min 12 sec
29	Row 1: Remove Excess	0 min 17 sec
30	Row 2: Cell Prefixation Master Mix	1 min 4 sec
31	Row 2: Strain Cells 1	0 min 12 sec
32	Row 2: Remove Excess	0 min 17 sec
33	Row 3: Cell Prefixation Master Mix	1 min 4 sec
34	Row 3: Strain Cells 1	0 min 12 sec
35	Row 3: Remove Excess	0 min 17 sec
36	Row 4: Cell Prefixation Master Mix	1 min 4 sec
37	Row 4: Strain Cells 1	0 min 12 sec
38	Row 4: Remove Excess	0 min 17 sec
39	Row 5: Cell Prefixation Master Mix	1 min 3 sec
40	Row 5: Strain Cells 1	0 min 12 sec
41	Row 5: Remove Excess	0 min 17 sec
42	Row 6: Cell Prefixation Master Mix	1 min 3 sec

STEPS	ACTION	DURATION
43	Row 6: Strain Cells 1	0 min 12 sec
44	Row 6: Remove Excess	0 min 17 sec
45	Row 7: Cell Prefixation Master Mix	1 min 3 sec
46	Row 7: Strain Cells 1	0 min 12 sec
47	Row 7: Remove Excess	0 min 17 sec
48	Row 8: Cell Prefixation Master Mix	1 min 3 sec
49	Row 8: Strain Cells 1	0 min 12 sec
50	Row 8: Remove Excess	0 min 17 sec
51	Prompt to tap plate	0
52	Prompt to remove strainer	0
53	Prompt to discard plate on Deck B	0
54	Add Fixation Solution	5 min 37 sec
55	Fixation Soln Incubation	5 min 0 sec
56	Add Perm Solution	5 min 34 sec
57	Add Fix and Perm Stop Buffer	6 min 3 sec
58	Row 1: Remove SN 1	0 min 22 sec
59	Row 1: Remove SN 2	0 min 15 sec
60	Row 1: Remove SN 3	0 min 19 sec
61	Row 2: Remove SN 1	0 min 22 sec
62	Row 2: Remove SN 2	0 min 15 sec
63	Row 2: Remove SN 3	0 min 19 sec
64	Row 3: Remove SN 1	0 min 23 sec
65	Row 3: Remove SN 2	0 min 15 sec
66	Row 3: Remove SN 3	0 min 18 sec

STEPS	ACTION	DURATION
67	Row 4: Remove SN 1	0 min 22 sec
68	Row 4: Remove SN 2	0 min 15 sec
69	Row 4: Remove SN 3	0 min 18 sec
70	Row 5: Remove SN 1	0 min 22 sec
71	Row 5: Remove SN 2	0 min 14 sec
72	Row 5: Remove SN 3	0 min 18 sec
73	Row 6: Remove SN 1	0 min 23 sec
74	Row 6: Remove SN 2	0 min 15 sec
75	Row 6: Remove SN 3	0 min 18 sec
76	Row 7: Remove SN 1	0 min 22 sec
77	Row 7: Remove SN 2	0 min 15 sec
78	Row 7: Remove SN 3	0 min 18 sec
79	Row 8: Remove SN 1	0 min 22 sec
80	Row 8: Remove SN 2	0 min 15 sec
81	Row 8: Remove SN 3	0 min 18 sec
82	Row 1: Cell Storage Master Mix	0 min 33 sec
83	Row 1: Resusp 1	0 min 18 sec
84	Row 1: Resusp 2	0 min 18 sec
85	Row 1: Strain Cells 1	0 min 41 sec
86	Row 1: Remove Excess	0 min 17 sec
87	Row 2: Cell Storage Master Mix	0 min 33 sec
88	Row 2: Resusp 1	0 min 18 sec
89	Row 2: Resusp 2	0 min 18 sec
90	Row 2: Strain Cells 1	0 min 41 sec

STEPS	ACTION	DURATION
91	Row 2: Remove Excess	0 min 17 sec
92	Row 3: Cell Storage Master Mix	0 min 33 sec
93	Row 3: Resusp 1	0 min 18 sec
94	Row 3: Resusp 2	0 min 18 sec
95	Row 3: Strain Cells 1	0 min 41 sec
96	Row 3: Remove Excess	0 min 17 sec
97	Row 4: Cell Storage Master Mix	0 min 33 sec
98	Row 4: Resusp 1	0 min 18 sec
99	Row 4: Resusp 2	0 min 18 sec
100	Row 4: Strain Cells 1	0 min 41 sec
101	Row 4: Remove Excess	0 min 17 sec
102	Row 5: Cell Storage Master Mix	0 min 33 sec
103	Row 5: Resusp 1	0 min 18 sec
104	Row 5: Resusp 2	0 min 18 sec
105	Row 5: Strain Cells 1	0 min 41 sec
106	Row 5: Remove Excess	0 min 17 sec
107	Row 6: Cell Storage Master Mix	0 min 33 sec
108	Row 6: Resusp 1	0 min 18 sec
109	Row 6: Resusp 2	0 min 18 sec
110	Row 6: Strain Cells 1	0 min 41 sec
111	Row 6: Remove Excess	0 min 17 sec
112	Row 7: Cell Storage Master Mix	0 min 33 sec
113	Row 7: Resusp 1	0 min 18 sec
114	Row 7: Resusp 2	0 min 18 sec

STEPS	ACTION	DURATION
115	Row 7: Strain Cells 1	0 min 41 sec
116	Row 7: Remove Excess	0 min 17 sec
117	Row 8: Cell Storage Master Mix	0 min 33 sec
118	Row 8: Resusp 1	0 min 18 sec
119	Row 8: Resusp 2	0 min 18 sec
120	Row 8: Strain Cells 1	0 min 41 sec
121	Row 8: Remove Excess	0 min 17 sec
122	Prompt to tap plate.	0
123	Prompt to remove the cell strainer.	0
124	Prompt to remove labware.	0
125	Prompt to move labware	0
126	Create Counting Aliquot Plate	4 min 48 sec
127	Create Sample Storage Plate	4 min 45 sec

Appendix B: Revision History

Version	Description	Date
1.0	Initial release	June 2026

PARSE BIOSCIENCES

A QIAGEN company

parsebiosciences.com

support@parsebiosciences.com

Scan code to visit site