User Manual

Version 1.2 - UMFC3300INT

Evercode™ Cells and Nuclei Fixation v3 with INTEGRA ASSIST PLUS

For use with

ECFC3300, ECFC3301
ECFC3303, ECFC3305
ECFC3501, ECFC3503
ECFC3505, ECFN3300
ECFN3301, ECFN3501

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U.S. Pat. No. 10,900,065

U.S. Pat. No. 11,168,355

U.S. Pat. No. 11,427,856

U.S. Pat. No. 11,634,751

U.S. Pat. No. 11,639,519

U.S. Pat. No. 11,680,283

Patents pending in the U.S. and other countries

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Table of Contents

Overview 4

Workflow 4

Important Guidelines 6

Cell Fixation Reagents 11

Nuclei Fixation Reagents 15

Parse-Provided Equipment 17

INTEGRA Components 18

INTEGRA-Provided Consumables 18

Consumables 19

Section 1: Set Up 20

Section 1.1. Block Plates with BSA 20

Section 1.2. Prepare Master Mixes for Cell Fixation 22

Section 1.3. Prepare Master Mixes for Nuclei Fixation 26

Section 2: Fixation 30

Section 2.1. 12 Reactions Fixation 30

Section 2.2. 48/96 Reactions Fixation 37

Appendices 45

Appendix A: Pipetting Programs 45

Appendix B: Revision History 53

Overview

Workflow

The Evercode Cells and Nuclei Fixation workflow is now 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 and Nuclei Fixation kits generate fixed and permeabilized cells/nuclei ready for use in all downstream Evercode assays.

Three different throughput options provide the flexibility to process anywhere between 1 to 96 samples in parallel:

- 12 Reactions workflows uses ECFC3300/ECFN3300
- 48 Reactions workflows uses half of ECFC3501/ECFN3501
- 96 Reactions workflows uses ECFC3501/ECFN3501

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.

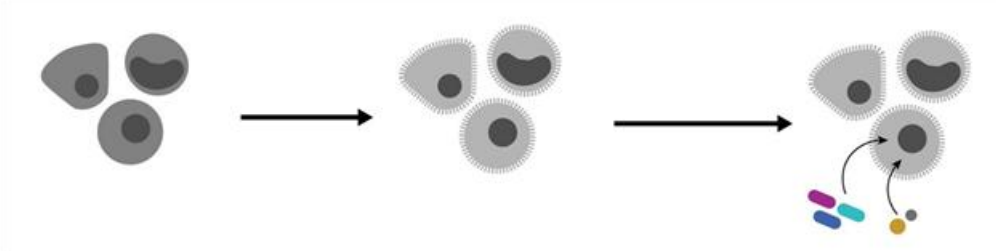


Figure 1: Evercode Cell Fixation. Cells in suspension are fixed and permeabilized before undergoing the split-pool combinatorial barcoding steps.

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 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.

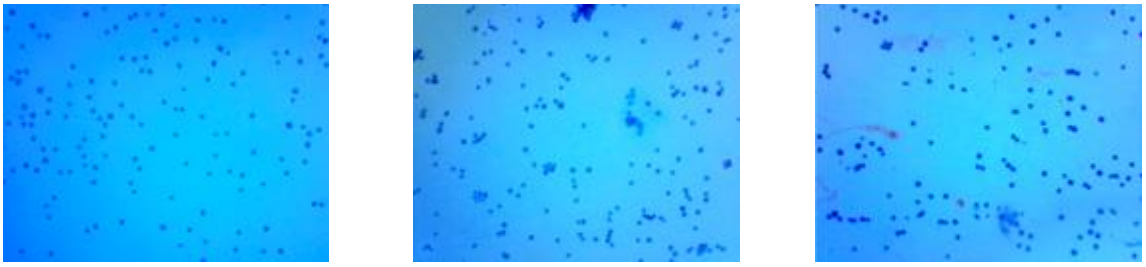
Plate Strainers

- An example video of using a Plate Strainer can be found in our support site. We recommend watching this video and practicing using Plate Strainers before processing your samples.
- After opening their plastic sleeves, Plate Strainers should be stored in their original plastic sleeve until use and used on the same day they were opened. We do not recommend using Plate Strainers more than one day after the plastic sleeve has been opened.
- Plate Strainers may need to be cut prior to processing samples. Plate Strainers should be cut with sterile scissors, razor blade, or scalpel that have been cleaned with RNaseZap RNase Decontamination Solution (Thermo Fisher Scientific) immediately prior to use.
- Plate Strainers should be thoroughly adhered to 96 well plates prior to use. After being placed by hand, a plate seal applicator should be used to completely seal the strainer over each well by pressing along the outside of the wells. The plate seal applicator should be cleaned with RNaseZap RNase Decontamination Solution (Thermo Fisher Scientific) immediately prior to use. Applicators should never be pressed directly onto the mesh.
- To maximize cell/nuclei retention with Plate Strainers, press pipette tips directly against the mesh. Ensure ample pressure is applied to hold contact between the tip and the strainer to force liquid through in ~1 second.

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 v3 kits.
- When first using Evercode Fixation v3 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



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 v3 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 4 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 tube strip(s).

PCR Freezer Block

- The PCR freezer block changes color from dark purple to bright pink (too warm) when the block temperature exceeds 7°C. If 70% of the block is bright pink, replace the PCR freezer block with a fully frozen dark purple block.
- To maintain optimal performance and minimize temperature fluctuations, store the PCR Freezer Blocks in a -20°C freezer when not in use.
- Tip pinching may occur when using a fully frozen PCR freezer block for a plate pooling step. To minimize tip pinching, a fully frozen PCR freezer 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 freezer block, apply firm pressure on the PCR plates until they are securely seated. Avoid touching the top of the open wells.

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 v3, 12 reactions workflow requires Cell Fixation Reagents and Cell Fixation Enhancer boxes.

Cell Fixation Reagents, 12 reactions. Store at -20°C, PN CF100

LABEL	ITEM	PN	FORMAT	QTY
	Prefixation Buffer	CF101	8 mL bottle	1
	Storage Buffer	CF102	2 mL tube	1
	Fixative Solution A	CF103	1.5 mL tube	1
	Fixative Solution B	CF104	1.5 mL tube	1
	Permeabilization Solution	CF105	1.5 mL tube	1
	Fix and Perm Stop Buffer	CF106	8 mL bottle	1
	RNase Inhibitor	CF107	1.5 mL tube	1
	DMSO	CF108	1.5 mL tube	1

Cell Prefixation Enhancer, 12 reactions. Store at 4°C, PN CF400

LABEL	ITEM	PN	FORMAT	QTY
	Prefixation Enhancer	CF201	1.5 mL tube	1

30 µM Plate Strainer*. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Adhesive Plate Strainer, 30 µM	PS030	Plastic sleeve	1

70 µM Plate Strainer*. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Adhesive Plate Strainer, 70 µM	PS070	Plastic sleeve	1

100 µM Plate Strainer*. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Adhesive Plate Strainer, 100 µM	PS100	Plastic sleeve	1

Note: * Only one mesh size of Plate Strainer is required for the Evercode Cell Fixation v3 workflow. Select an appropriate mesh size for each sample type.

The Evercode Cell Fixation v3, 96 reactions workflow requires Cell Fixation Reagents and Cell Fixation Enhancer boxes.

Cell Fixation Reagents, 96 reactions. Store at -20°C, PN CF300

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	CF305	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 CF400

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

30 µM Plate Strainer*. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Adhesive Plate Strainer, 30 µM	PS030	Plastic sleeve	2

70 µM Plate Strainer*. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Adhesive Plate Strainer, 70 µM	PS070	Plastic sleeve	2

100 µM Plate Strainer*. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Adhesive Plate Strainer, 100 µM	PS100	Plastic sleeve	2

Note: * Only one mesh size of Plate Strainer is required for the Evercode Cell Fixation v3 workflow. Select an appropriate mesh size for each sample type.

Nuclei Fixation Reagents

The Evercode Nuclei Fixation v3, 12 reactions workflow requires Nuclei Fixation Reagents and Nuclei Fixation Enhancer boxes.

Nuclei Fixation Reagents, 12 reactions. Store at -20°C, NF100

LABEL	ITEM	PN	FORMAT	QTY
	Prefixation Buffer	NF101	8 mL bottle	1
	Storage Buffer	NF102	2 mL tube	1
	Fixative Solution	NF103	1.5 mL tube	1
	Permeabilization Solution	NF104	1.5 mL tube	1
	Fix and Perm Stop Buffer	NF105	8 mL bottle	1
	RNase Inhibitor	NF106	1.5 mL tube	1
	DMSO	NF107	1.5 mL tube	1

Nuclei Fixation Enhancer, 12 reactions. Store at 4°C, NF200

LABEL	ITEM	PN	FORMAT	QTY
	Prefixation Enhancer	NF201	1.5 mL tube	1

30 µm Plate Strainer. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Adhesive Plate Strainer, 30 µM	PS030	Plastic sleeve	1

The Evercode Nuclei Fixation v3, 96 reactions workflow requires Nuclei Fixation Reagents and Nuclei Fixation Enhancer boxes.

Nuclei Fixation Reagents, 96 reactions. Store at -20°C, NF300

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	NF304	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, NF400

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

30 µm Plate Strainer. Store at Room Temperature

LABEL	ITEM	PN	FORMAT	QTY
N/A	Adhesive Plate Strainer, 30 µM	PS030	Plastic sleeve	2

Parse-Provided 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
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
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-Provided Consumables

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

ITEM	ITEM TYPE	PN	QTY
8 Row Reagent Reservoirs, Partitioned (32 mL/row) with SUREFLO design	INTEGRA-Provided	6373	4
10-300 µL pipette tips (Sterile/Filter/Low Retention)	INTEGRA-Provided	6535	1

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	QTY
Semi skirted plates (clear)	Thermo Fisher Scientific®	E951020362	2
1 mL Deepwell Plate	Eppendorf	951033308	2

Equipment

ITEM	SUPPLIER	PN	NOTES
Microcentrifuge	Various Suppliers	Varies	Compatible with 1.5 mL tubes.
Hemocytometer	Sigma-Aldrich®	Z359629	Or other cell counting devices. We recommend validating alternatives relative to a hemocytometer.
Plate Seal Applicator	Various Suppliers	Varies	Capable of adhering plate sealing films to 96 well plates.
Trypan Blue	Various Suppliers	Varies	Or alternative dyes to assess cell viability, such as AO/PI.

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	12	48	96
Nuclease-free water (not supplied)	21.8 mL	87 mL	175 mL
Gibco Bovine Albumin Fraction V (7.5% solution) (not supplied)	3.4 mL	13 mL	27 mL
Total Volume	25.2 mL	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 or 2 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.

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

ITEM	SOURCE	QTY (12 or 48/96)	HANDLING AND STORAGE
8 Row Reservoir	INTEGRA-Provided	1	Individually wrapped consumable
1 mL 96 Deepwell plate	Eppendorf	1	
Fixation Cooling Adapter Base	Parse-Provided	1	
Fixation 8 Row Reservoir Adapter	Parse-Provided	1	
○ Prefixation Buffer	Cell Fixation Reagents (-20°C)	8 mL bottle or 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)	2 mL tube or 8 mL bottle	
○ Fixative Solution A	Cell Fixation Reagents (-20°C)	1.5 mL tube or 8 mL bottle	
○ Fixative Solution B	Cell Fixation Reagents (-20°C)	1.5 mL tube or 8 mL bottle	
● Permeabilization Solution	Cell Fixation Reagents (-20°C)	1.5 mL tube or 2 mL tube	
○ Fix and Perm Stop Buffer	Cell Fixation Reagents (-20°C)	8 mL bottle or 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.
● Prefixation Enhancer	Cell Fixation Reagents (4°C)	1.5 mL tube or 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	12	48	96
○ Prefixation Buffer	2.67 mL	10.7 mL	21.3 mL
● RNase Inhibitor	36 µL	144 µL	288 µL
● Prefixation Enhancer	180 µL	720 µL	1.4 mL
Total Volume	2.9 mL	11.5 mL	23 mL

CRITICAL! The overages in these master mixes are designed for use with the Evercode Cell Fixation v3, HT 96 reactions kit when processing ≥ 48 samples across up to 2 fixation sessions. If the kit is to be used more than twice, the mid throughput plate-based workflow should be used instead to ensure that there are sufficient reagent overages.

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	12	48	96
○ Fixative Solution A	526 µL	2.4 mL	4.8 mL
○ Fixative Solution B	526 µL	2.4 mL	4.8 mL
Total Volume	1.05 mL	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	12	48	96
○ Storage Buffer	1.732 mL	6.3 mL	12.6 mL
● RNase Inhibitor	23.1 µL	84 µL	168 µL
● DMSO	92.4 µL	336 µL	672 µL
Total Volume	1.848 mL	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. For the 12 Reactions Fixation only: add **45 µL** of ● Permeabilization Solution into each well of Row B of the sample 96 Deepwell plate.

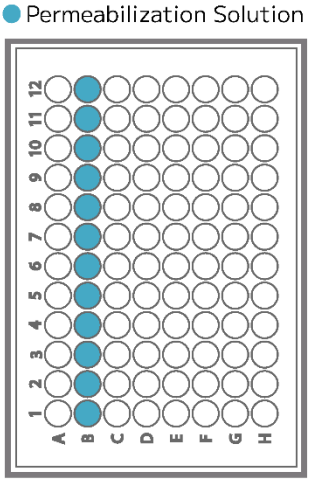
PERMEABILIZATION SOLUTION		
Number of Samples	48	96
● Permeabilization Solution	~2 mL	~4 mL

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.

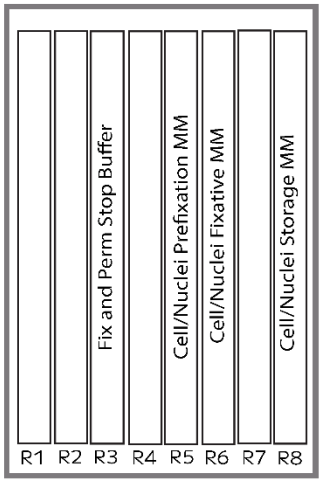
FIX AND PERM STOP BUFFER			
Number of Samples	12	48	96
○ Fix and Perm Stop Buffer	~3.5 mL	~14 mL	~28 mL

7. The 8 Row Reservoir should correspond to Reagent 8 Row Reservoir represented below.

For 12 Cell Fixation Reactions

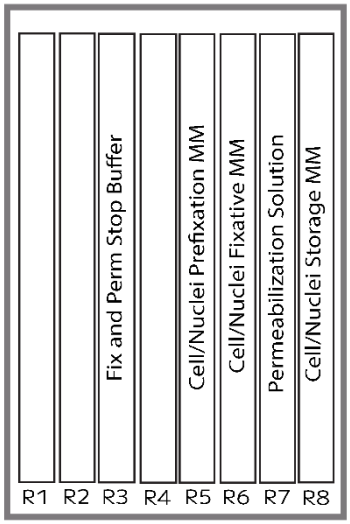


Sample Plate



8 Row Reservoir

For 48/96 Cell Fixation Reactions



8 Row Reservoir

CRITICAL! Ensure that the liquid is distributed evenly throughout the reservoir.

Section 1.3. Prepare Master Mixes for Nuclei Fixation

Nuclei fixation master mixes should be prepared just prior to fixation.

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

ITEM	SOURCE	QTY (12 or 48/96)	HANDLING AND STORAGE
8 Row Reservoir	INTEGRA-Provided	1	Individually wrapped consumable
1 mL 96 Deepwell plate	Eppendorf	1	
Fixation Cooling Adapter Base	Parse-Provided	1	
Fixation 8 Row Reservoir Adapter	Parse-Provided	1	
○ Prefixation Buffer	Nuclei Fixation Reagents (-20°C)	8 mL bottle or 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)	2 mL tube or 8 mL bottle	
○ Fixative Solution	Nuclei Fixation Reagents (-20°C)	1.5 mL tube or 8 mL bottle	
● Permeabilization Solution	Nuclei Fixation Reagents (-20°C)	1.5 mL tube or 2 mL tube	
○ Fix and Perm Stop Buffer	Nuclei Fixation Reagents (-20°C)	8 mL bottle or 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)	1.5 mL tube or 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	12	48	96
○ Prefixation Buffer	2.53 mL	10.7 mL	21.3 mL
● RNase Inhibitor	34.79 µL	144 µL	288 µL
● Prefixation Buffer	173.94 µL	720 µL	1.4 mL
Total Volume	2.7 mL	11.5 mL	23 mL

The overages in these master mixes are designed for use with the Evercode Nuclei Fixation v3, HT 96 reactions kit when processing ≥ 48 samples across up to 2 fixation sessions. If the kit is to be used more than twice, the mid throughput plate-based workflow should be used instead to ensure that there are sufficient reagent overages.

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

NUCLEI FIXATIVE SOLUTION			
Number of Samples	12	48	96
○ Fixative Solution	~1 mL	~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	12	48	96
○ Storage Buffer	1.732 mL	6.3 mL	12.6 mL
● RNase Inhibitor	23.1 µL	84 µL	168 µL
● DMSO	92.4 µL	336 µL	672 mL
Total Volume	1.848 mL	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. For the 12 Reactions Fixation only: add **45 µL** of ● Permeabilization Solution into each well of Row B of the sample 96 Deepwell Plate.

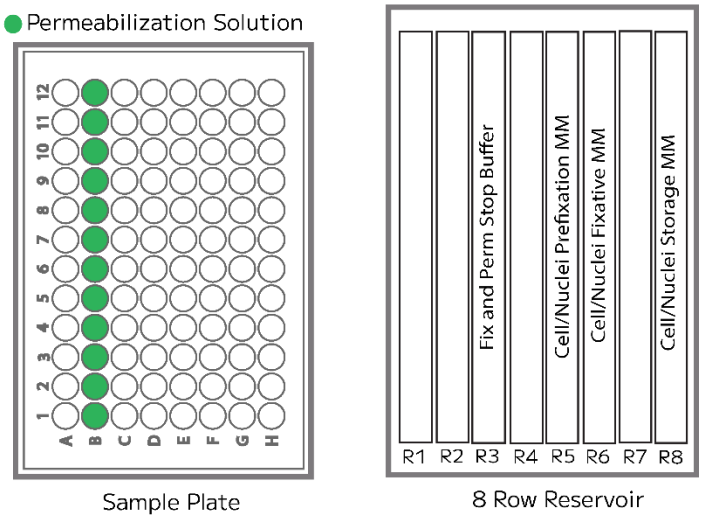
PERMEABILIZATION SOLUTION		
Number of Samples	48	96
● Permeabilization Solution	~2 mL	~4 mL

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.

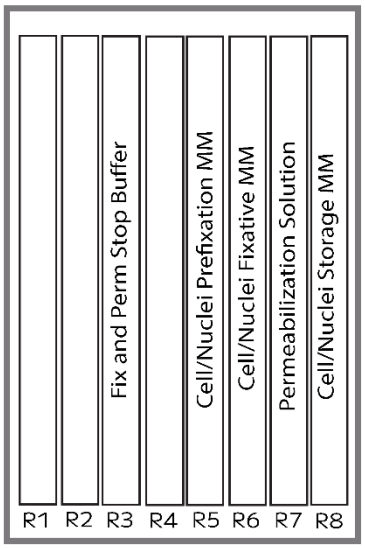
FIX AND PERM STOP BUFFER			
Number of Samples	12	48	96
○ Fix and Perm Stop Buffer	~3.5 mL	~14 mL	~28 mL

7. The 8 Row Reservoir should correspond to the Reagents 8 Row reservoir represented below.

For 12 Nuclei Fixation Reactions



For 48/96 Nuclei Fixation Reactions



8 Row Reservoir

CRITICAL! Ensure that the liquid is distributed evenly throughout the reservoir.

Section 2: Fixation

Section 2.1. 12 Reactions Fixation

To fix samples:

1. Gather the following components:

ITEM	SOURCE	QTY	HANDLING AND STORAGE
VIAFLO Pipette 12-Ch, 10-300 µL	INTEGRA Component	N/A	
Tip Deck for VIAFLO Pipetting Module	INTEGRA Component	N/A	
10-300 µL Tip Rack	INTEGRA-Provided	1	
Thermochromic PCR Cold Block	Parse-Provided	1	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	1	
Fixation Deep Well Adapter	Parse-Provided	1	
Fixation Cooling Adapter Base	Parse-Provided	1	

2. Cool the centrifuge with a swinging bucket rotor to 4°C.
3. Fill a bucket with ice.
4. Prepare a hemocytometer, flow cytometer, or other cell counting device.
5. Count the cells in the single cell suspension with a hemocytometer or alternative counting device and record the count. Keep cells on ice during counting and work quickly to minimize time on ice prior to fixation.
6. With the plate on ice, transfer 100,000 to 1 million cells from each sample into the wells of a 1 mL Deepwell Plate (or BSA-coated deep well plate if prepared in Section 1.1). All samples should be in Row A.
7. Add a new plate seal on the deep well plate with the cell suspension.

8. 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.

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

9. Attach VIAFLO Pipetting Module 12-Ch, 10-300 µL and the corresponding Tip Deck.
10. Place a Thermochromic PCR Cold Block with Riser on Deck A.
11. Place a PCR semi-skirted plate on Deck A.
12. Fill two of the Cooling Adapter Bases with pebble ice and place one on 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 Adapter Bases were frozen overnight, refill the cooling base with pebble ice at the 90 minute mark.

13. Place the Fixation Deep Well Adapter on the Fixation Cooling Adapter Base on Deck B.
14. Place the Fixation 8 Row Reservoir Cooling Adapter on the Cooling Adapter Base on Deck C.
15. Place the 8 Row Reservoir with Reagents prepared Section 1 on the Fixation 8 Row Reservoir Adapter on Deck C with **row 1** facing the left side.
16. Once the 10 minute cell centrifugation is complete, place the 1 mL Deepwell Plate on top of the Fixation Deep Well Plate Adapter on Deck B and remove the plate seal (Figure 3).

17. Cut an individual row of the plate strainer. Adhere one row of the plate strainer on Row D of the Deepwell Plate.

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

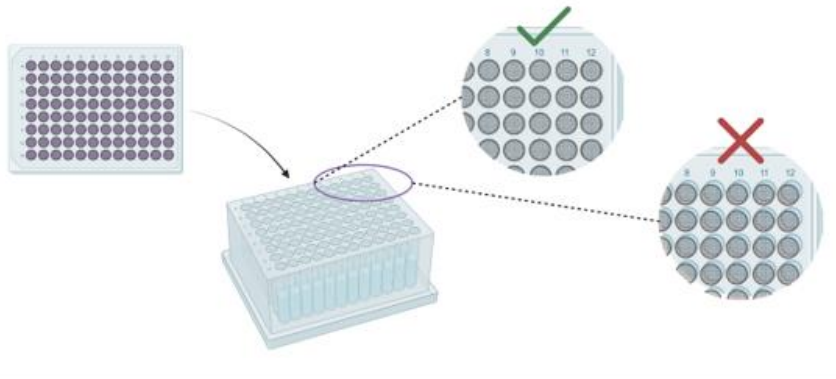
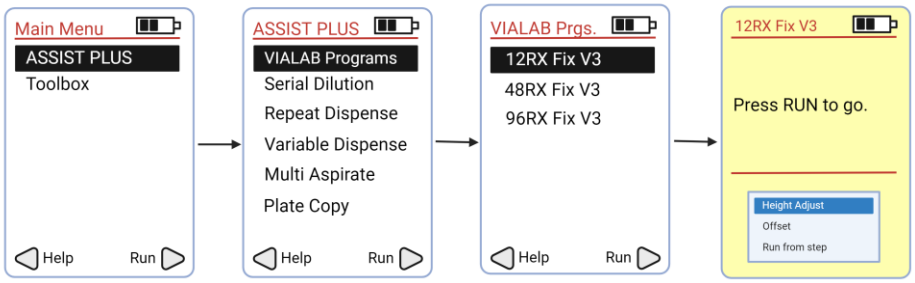


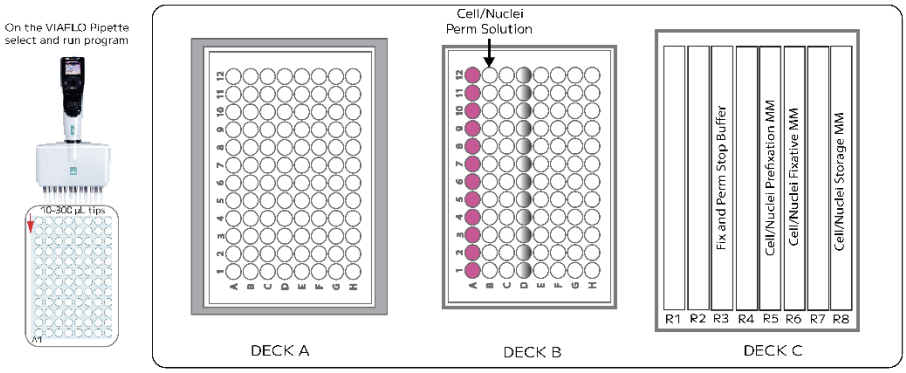
Figure 3: The tops of the strainer and the well openings line up to avoid sample loss.

18. Run the program **12RX Fix V3** following the diagram below.



19. Deck layout should correspond to the configuration below.

Deck Configuration



	DECK A	DECK B	DECK C
HARDWARE	Thermochromic PCR Cold Block Thermochromic PCR Cold Block Riser	Fixation Cooling Adapter Base Fixation Deepwell Adapter	Fixation Cooling Adapter Base Fixation 8 Row Reservoir Cooling Adapter
CONSUMABLES	Eppendorf Semi-skirted plate	96 Deepwell Plate plate strainer (**Row D**)	8 Row Reservoir
REAGENTS		Permeabilization Solution (**Row B**) ● Samples (**Row A**)	**R3**: Fix and Perm Stop Buffer **R5**: Cell Prefixation Master Mix **R6**: Cell Fixative Master Mix **R8**: Cell Storage Master Mix

20. **When prompted**, tap the plate on Deck B 3x on the deck to move liquid to the bottom of the wells.

21. **When prompted**, carefully remove the strainer on Row D of the 96 deep well plate on Deck B.

CRITICAL! Remove the strainer with one swift motion to reduce chances of contamination.

22. Press "Run" to continue.

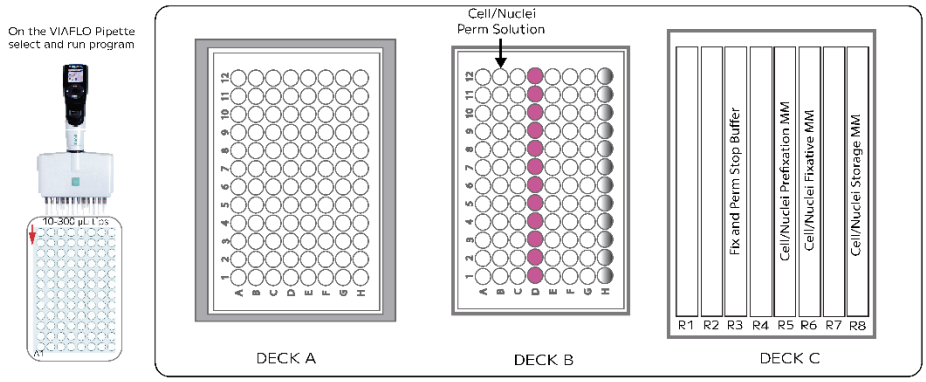
23. **When prompted**, seal and centrifuge the plate on Deck B in a swinging bucket rotor for **5-10 minutes** at 200-500 x g at 4°C.

24. Press "Run" to continue.

25. When the 10 minute centrifugation is complete, place the 1 mL Deepwell Plate on top of the Fixation Deepwell Adapter on Deck B and remove the plate seal.

26. Add a plate strainer on Row H. Deck layout should correspond to the configuration below.

Deck Configuration



	DECK A	DECK B	DECK C
HARDWARE	Thermochromic PCR Cold Block Thermochromic PCR Cold Block Riser	Fixation Cooling Adapter Base Fixation Deep Well Adapter	Fixation Cooling Adapter Base Fixation Row Reservoir Cooling Adapter
CONSUMABLES	Eppendorf Semi-skited plate	96 Deepwell Plate plate strainer (**Row H**)	8 Row Reservoir
REAGENTS		Permeabilization Solution (**Row B**) ● Samples (**Row D**)	**R3**: Fix and Perm Stop Buffer **R5**: Cell Prefixation Master Mix **R6**: Cell Fixative Master Mix **R8**: Cell Storage Master Mix

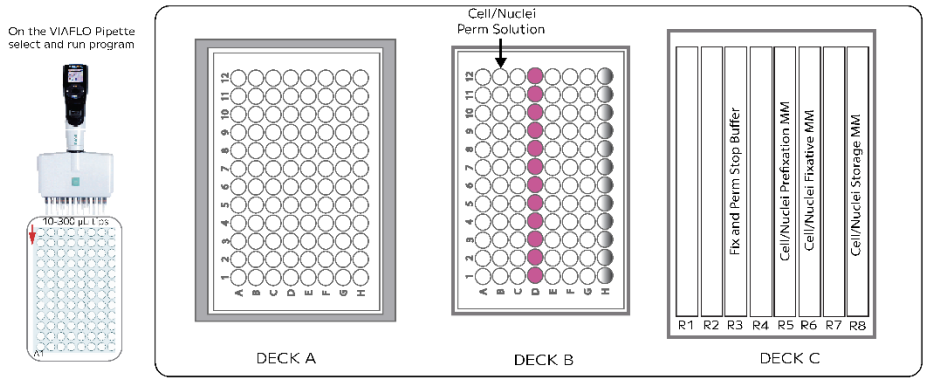
27. **When prompted,** tap the plate on Deck A 3x on the deck to move liquid to the bottom of the wells.

CRITICAL! Remove the strainer with one swift motion to reduce the chances of contamination.

28. **When prompted**, carefully peel off the plate strainer and discard it.

29. **When prompted**, replace the Eppendorf semi-skirted plate on deck A with a new Eppendorf semi-skirted plate on the Thermochromic PCR Cold Block on Deck A. The plate that was removed contains a counting aliquot. Store the plate on ice. Deck layout should correspond to the configuration below.

Deck Configuration



	DECK A	DECK B	DECK C
HARDWARE	Thermochromic PCR Cold Block Thermochromic PCR Cold Block Riser	Fixation Cooling Adapter Base Fixation Deepwell Adapter	Fixation Cooling Adapter Base Fixation 8 Row Reservoir Adapter
CONSUMABLES	Eppendorf semi-skirted plate	Eppendorf semi-skirted plate	8 Row Reservoir
REAGENTS		Permeabilization Solution (**Row B**) ● Samples (**Row H**)	**R3**: Fix and Perm Stop Buffer **R5**: Cell Prefixation Master Mix **R6**: Cell Fixative Master Mix **R8**: Cell Storage Master Mix

30. Press "Run" to continue the program.

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

31. After the program is complete, seal the semi-skirted plates with a seal that can withstand storage at -80°C or transfer the remainder of the sample to 0.2 mL tube strip(s). The first semi-skirted plate on Deck A contains a 20 µL counting aliquot. The second semi-skirted plate on Deck A contains the fixed cell suspension mix.

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

32. Place the samples in a room temperature styrofoam 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.

33. The day before running the downstream Evercode Whole Transcriptome kit, thaw the 20 µL aliquots in the semi-skirted plate in a water bath 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.

Section 2.2. 48/96 Reactions Fixation

To fix samples:

1. Gather the following components:

ITEM	SOURCE	QTY	HANDLING AND STORAGE
VIAFLO Pipette 12-Ch, 10-300 µL	INTEGRA Component	N/A	
Tip Deck for VIAFLO Pipetting Module	INTEGRA Component	N/A	
10-300 µL Tip Rack	INTEGRA-Provided	1	
Thermochromic PCR Cold Block	Parse-Provided	1	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	1	

2. Cool the centrifuge with a swinging bucket rotor to 4°C.
3. Fill a bucket with ice.
4. Prepare a hemocytometer, flow cytometer, or other cell counting device.
5. Count the cells in the single cell suspension with a hemocytometer or alternative counting device and record the count. Keep cells on ice during counting and work quickly to minimize time on ice prior to fixation.
6. With the plate on ice, transfer 100,000 to 1 million cells 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).
    a. Row A - Row D for 48 Samples
    b. Row A - Row H for 96 Samples
7. Add a new plate seal on the deep well plate with the cell suspension.

8. 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.

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

9. Attach VIAFLO Pipetting Module 12-Ch, 10-300 µL and the corresponding Tip Deck.

10. 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.

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

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

13. Place a new 8 Row Reservoir on the Fixation 8 Row Reservoir Adapter on Deck C.

14. Put a plate strainer on a new 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 (Figure 4).

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

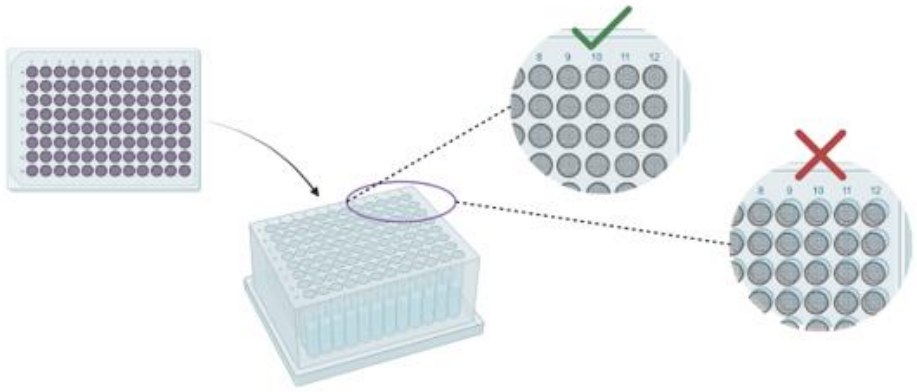
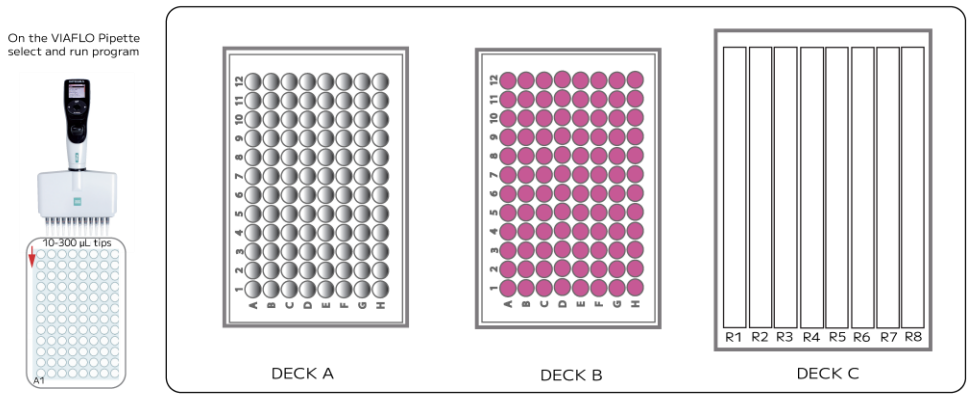


Figure 4: The tops of the strainer and the well openings line up to avoid sample loss.

15. Once the 10 minute centrifugation is complete, place the 1 mL deep well plate on top of the Fixation Deep Well Adapter on Deck B and remove the plate seal. Deck layout should correspond to the configuration below.

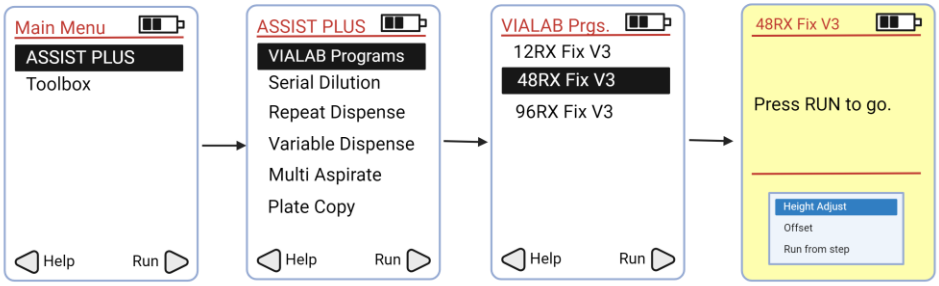
Deck Configuration



	DECK A	DECK B	DECK C
HARDWARE	Cooling Adapter Base Fixation Deep Well Adapter	Cooling Adapter Base Fixation Deep Well Adapter	Cooling Adapter Base Fixation 8 Row Reservoir Adapter

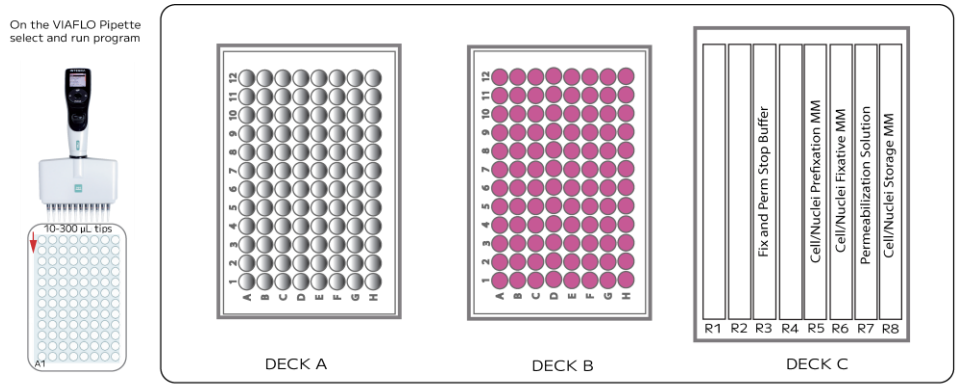
	DECK A	DECK B	DECK C
CONSUMABLES	96 deep well plate + plate cell strainer	96 Deepwell Plate	Empty 8 Row Reservoir
REAGENTS		● Samples	

16. Run the program **48RX Fix V3** if running up to 48 samples, or program **96RX Fix V3** if running up to 96 samples.



17. **When prompted**, replace the cell waste from Deck C with the Reagent 8 Row Reservoir prepared in Section 1. Deck layout should correspond to the configuration below.

Deck Configuration



	DECK A	DECK B	DECK C
HARDWARE	Cooling Adapter Base Fixation Deep Well Adapter	Cooling Adapter Base Fixation Deep Well Adapter	Cooling Adapter Base Fixation 8 Row Reservoir Adapter
CONSUMABLES	96 deep well plate + plate cell strainer	96 Deepwell Plate	8 Row Reservoir
REAGENTS		● Samples	**R3**: Fix and Perm Stop Buffer **R5**: Cell Prefixation Master Mix **R6**: Cell Fixative Master Mix **R7**: Permeabilization Solution **R8**: Cell Storage Master Mix

18. **When prompted,** discard the 96 deep well plate on Deck B.

19. **When prompted**, tap the plate on Deck A 3x on the deck to move liquid to the bottom of the wells.

20. **When prompted**, carefully remove the strainer on the 96 deep well plate on Deck A.

CRITICAL! Remove the strainer with one swift motion to reduce the chances of contamination.

21. Press "Run" to continue.

22. **When prompted**, 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.

23. Press "Run" to continue.

24. **When prompted**, apply a 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 Deepwell Plate Cooling Adapter on Deck A with A1 towards the bottom left (Figure 5).

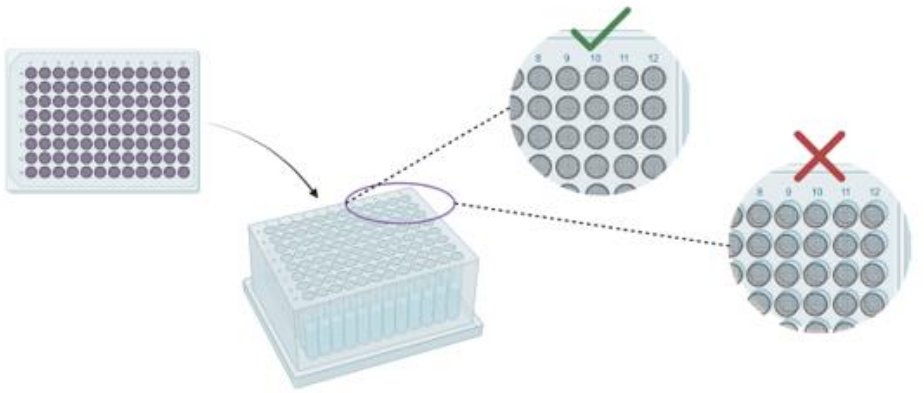
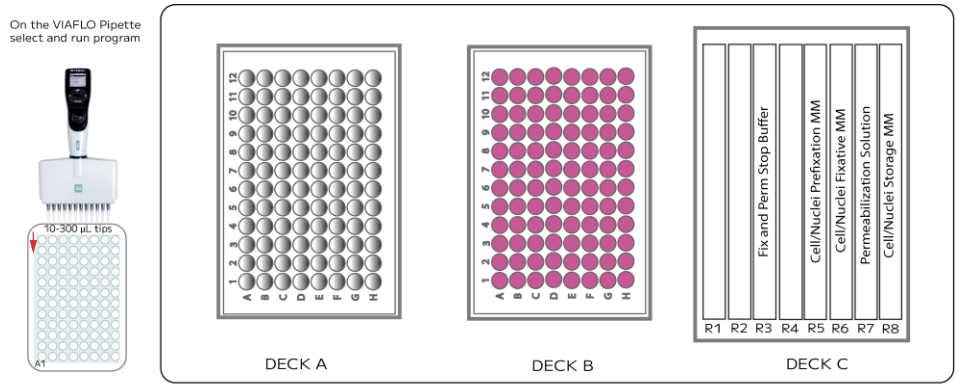


Figure 5: The tops of the strainer and the well openings line up to avoid sample loss.

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

25. Once the 10 minute centrifugation is complete, place the 1 mL Deepwell Plate on top of the Eppendorf 1mL Deepwell Plate Cooling Adapter on Deck B and remove the plate seal. Deck layout should correspond to the configuration below.

Deck Configuration



	DECK A	DECK B	DECK C
HARDWARE	Cooling Adapter Base Fixation Deep Well Adapter	Cooling Adapter Base Fixation Deep Well Adapter	Cooling Adapter Base Fixation 8 Row Reservoir Adapter
CONSUMABLES	96 deep well plate + plate cell strainer	96 Deepwell Plate	8 Row Reservoir

	DECK A	DECK B	DECK C
REAGENTS		● Samples	**R3**: Fix and Perm Stop Buffer **R5**: Cell Prefixation Master Mix **R6**: Cell Fixative Master Mix **R7**: Permeabilization Solution **R8**: Cell Storage Master Mix

26. **When prompted**, tap the plate on Deck A 3x on the deck to move liquid to the bottom of the wells.

27. **When prompted**, carefully peel off the plate strainer and discard.

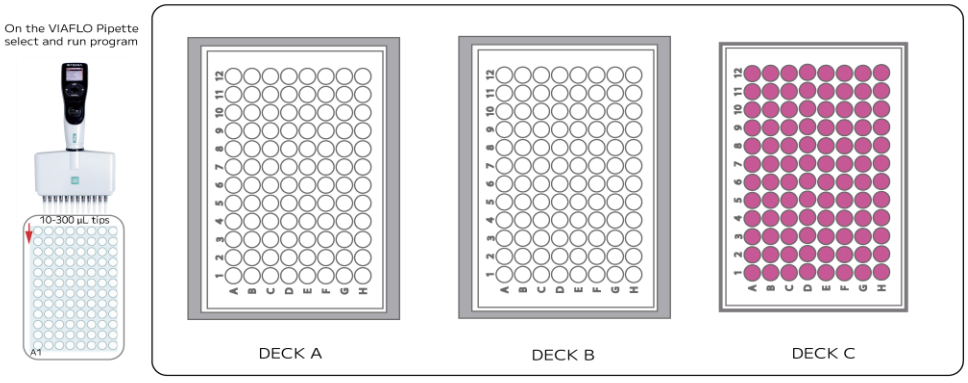
CRITICAL! Remove the strainer with one swift motion to reduce chances of contamination

28. **When prompted**, remove all labware on Decks B and C.

29. **When prompted**, move all labware from Deck A to Deck C.

30. **When prompted**, place 2 Thermochromic PCR Cold Blocks and new Eppendorf semi-skirted plates on the Thermochromic PCR Cold Blocks on both Decks A and B. Deck layout should correspond to the configuration below.

Deck Configuration



	DECK A	DECK B	DECK C
HARDWARE	Thermochromic PCR Cold Block	Thermochromic PCR Cold Block	Cooling Adapter Base Fixation Deep Well Adapter
CONSUMABLES	Eppendorf semi-skirted plate	Eppendorf semi-skirted plate	96 deep well plate
REAGENTS			● Samples

31. Press "Run" to continue the program.

32. After the program is complete, seal the semi-skirted plates on Deck A and B with a seal that can withstand storage at -80°C or transfer the remainder of the sample to 0.2 mL tube strip(s). The semi-skirted plate on Deck A contains a 20 µL counting aliquot. The semi-skirted plate on Deck B contains the fixed cell suspension mix.

Note: The 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 fixes samples in PCR plates.

33. Place the samples in a room temperature styrofoam 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.

34. The day before running the downstream Evercode Whole Transcriptome kit, thaw the 20 µL aliquots in the semi-skirted plate in a water bath 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

12_RXN_FIX V3

STEPS	ACTION
1	Initial Volumes
2-4	Row 1: Remove Supernatant
5	Virtual Volume Modification
6	Row 1: Add Cell Prefixation Buffer
7	Row 1: Strain
8	Row 1: Remove Excess
9	Prompt to tap plate
10	Prompt to remove strainer.
11	Add Fixation Solution
12	9.5 min Incubation
13	Add Perm Solution
14	2.5 min Incubation
15	Add Stop Mix
16	Prompt to seal and spin plate.
17	Prompt to place a plate on Deck B.
18	Prompt to add strainer.
19	Prompt to check the plate.
20	Virtual volume modification
21-23	Row 1: Remove Supernatant
24	Row 1: Add Cell Storage Master Mix

STEPS	ACTION
25	Row 1: Strain
26	Row 1: Remove Excess
27	Prompt to tap plate
28	Prompt to remove strainer.
29	Aliquot counting aliquots.
30	Sample Plate Notification
31	Prompt to replace PCR plate
32	Aliquot counting aliquots.
33	Sample Plate Notification

48 _RXN_FIX V3

STEPS	ACTION
1	Initial Volumes
2-4	Row 1: Remove Supernatant
5-7	Row 2: Remove Supernatant
8-10	Row 3: Remove Supernatant
11-13	Row 4: Remove Supernatant
14	Prompt to change the reservoir
15	Virtual volume modification
16	Row 1: Add Cell Prefixation Master Mix
17	Row 1: Strain Cells 1
18	Row 1: Remove Excess
19	Row 2: Add Cell Prefixation Master Mix
20	Row 2: Strain Cells 1
21	Row 2: Remove Excess

STEPS	ACTION
22	Row 3: Add Cell Prefixation Master Mix
23	Row 3: Strain Cells 1
24	Row 3: Remove Excess
25	Row 4: Add Cell Prefixation Master Mix
26	Row 4: Strain Cells 1
27	Row 4: Remove Excess
28	Prompt to tap plate
29	Prompt to remove strainer
30	Prompt to throw away the Deck B plate
31	Add Fixation Solution
32	8 Min Incubation
33	Add Perm Solution
34	30 Second Incubation
35	Add Fix and Perm Stop Buffer
36	Prompt to seal and spin plate
37	Prompt to add a strain plate on Deck A
38	Prompt to place cell suspension plate on Deck B.
39	Virtual volume modification
40-42	Row 1: Remove Supernatant
43-45	Row 2: Remove Supernatant
46-48	Row 3: Remove Supernatant
49-51	Row 4: Remove Supernatant
52	Row 1: Cell Storage Master Mix
53	Row 1: Strain Cells 1

STEPS	ACTION
54	Row 1: Remove Excess
55	Row 2: Cell Storage Master Mix
56	Row 2: Strain Cells 1
57	Row 2: Remove Excess
58	Row 3: Cell Storage Master Mix
59	Row 3: Strain Cells 1
60	Row 3: Remove Excess
61	Row 4: Cell Storage Master Mix
62	Row 4: Strain Cells 1
63	Row 4: Remove Excess
64	Prompt to tap plate.
65	Prompt to remove the cell strainer.
66	Prompt to remove labware.
67	Prompt to move labware
68	Prompt to add PCR cold blocks.
69	Prompt to add new PCR plates.
70	Labware Change
71	Create Counting Aliquot Plate
72	Create Sample Storage Plate

96_RXN_FIX V3

STEPS	ACTION
1	Initial Volumes
2-4	Row 1: Remove Supernatant
5-7	Row 2: Remove Supernatant

STEPS	ACTION
8-10	Row 3: Remove Supernatant
11-13	Row 4: Remove Supernatant
14-16	Row 5: Remove Supernatant
17-19	Row 6: Remove Supernatant
20-22	Row 7: Remove Supernatant
23-25	Row 8: Remove Supernatant
26	Prompt to change the reservoir
27	Virtual volume modification
28	Row 1: Add Cell Prefixation Master Mix
29	Row 1: Strain Cells 1
30	Row 1: Remove Excess
31	Row 2: Add Cell Prefixation Master Mix
32	Row 2: Strain Cells 1
33	Row 2: Remove Excess
34	Row 3: Add Cell Prefixation Master Mix
35	Row 3: Strain Cells 1
36	Row 3: Remove Excess
37	Row 4: Add Cell Prefixation Master Mix
38	Row 4: Strain Cells 1
39	Row 4: Remove Excess
40	Row 5: Add Cell Prefixation Master Mix
41	Row 5: Strain Cells 1
42	Row 5: Remove Excess
43	Row 6: Add Cell Prefixation Master Mix

STEPS	ACTION
44	Row 6: Strain Cells 1
45	Row 6: Remove Excess
46	Row 7: Add Cell Prefixation Master Mix
47	Row 7: Strain Cells 1
48	Row 7: Remove Excess
49	Row 8: Add Cell Prefixation Master Mix
50	Row 8: Strain Cells 1
51	Row 8: Remove Excess
52	Prompt to tap plate
53	Prompt to remove strainer
54	Prompt to throw away the Deck B plate
55	Add Fixation Solution
56	5 Min Incubation
57	Add Perm Solution
58	Add Fix and Perm Stop Buffer
59	Prompt to seal and spin plate
60	Prompt to add a strain plate on Deck A
61	Prompt to place cell suspension plate on Deck B.
62	Virtual volume modification
63-65	Row 1: Remove Supernatant
66-68	Row 2: Remove Supernatant
69-71	Row 3: Remove Supernatant
72-74	Row 4: Remove Supernatant
75-77	Row 5: Remove Supernatant

STEPS	ACTION
78-80	Row 6: Remove Supernatant
81-83	Row 7: Remove Supernatant
84-86	Row 8: Remove Supernatant
87	Row 1: Cell Storage Master Mix
88	Row 1: Strain Cells 1
89	Row 1: Remove Excess
90	Row 2: Cell Storage Master Mix
91	Row 2: Strain Cells 1
92	Row 2: Remove Excess
93	Row 3: Cell Storage Master Mix
94	Row 3: Strain Cells 1
95	Row 3: Remove Excess
96	Row 4: Cell Storage Master Mix
97	Row 4: Strain Cells 1
98	Row 4: Remove Excess
99	Row 5: Cell Storage Master Mix
100	Row 5: Strain Cells 1
101	Row 5: Remove Excess
102	Row 6: Cell Storage Master Mix
103	Row 6: Strain Cells 1
104	Row 6: Remove Excess
105	Row 7: Cell Storage Master Mix
106	Row 7: Strain Cells 1
107	Row 7: Remove Excess

STEPS	ACTION
108	Row 8: Cell Storage Master Mix
109	Row 8: Strain Cells 1
110	Row 8: Remove Excess
111	Prompt to tap plate.
112	Prompt to remove the cell strainer.
113	Prompt to remove labware.
114	Prompt to move labware
115	Prompt to add PCR cold blocks.
116	Prompt to add new PCR plates.
117	Labware Change
118	Create Counting Aliquot Plate
119	Create Sample Storage Plate

Appendix B: Revision History

Version	Description	Date
1.0	Initial release	October 2024
1.1	Updated pipette references in deck configurations	October 2024
1.2	Updated 12 Reactions Fixation deck configurations Updated copyrights date	January 2025

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