VisionSort Starter Kit

rev.1/25

Cat# TCK001

I.   INTRODUCTION

ThinkCyte's VisionSort platform combines the strengths of conventional flow cytometry fluorescence signals with a novel morphometric cellular analysis measure. The dual-mode analytical capability may be used to identify and sort phenotypically defined cell populations, label-free, using machine learning approaches. The Vision Starter Kit provides all the essential items (consumables, reagents) required for quick Start using the instrument.

II.   MATERIALS PROVIDED

No	Item	Catalog Number	Volume/Units	Storage Temp*	Handling Instructions**
1	VS Sort Cartridge	TCC-CPS001-10	10 Cartridges	RT	Do not drop; Light and Heat sensitive
2	VS Wash Cartridge	TCC-CPW001-1	2 Cartridges	RT	Do not drop; Light and Heat sensitive
3	15 mL Tubes	TCC-TU001-15-25	2 x 25 Tubes	RT	NA
4	50 mL Tubes	TCC-TU001-50-25	1 x 25 Tubes	RT	NA
5	VS Sheath	TCR-SH001-250	250 mL	RT	NA
6	VS Suspension Solution	TCR-SS001-100	100 mL	4°C	Heat sensitive
7	VS Clean	TCR-CN001-250	250 mL	RT	Light sensitive
8	VS C-Beads	TCB-CB001-1	100 µL	4°C	Light sensitive
9	VS P-RBW Beads	TCB-RBW001-1	200 µL	4°C	Light sensitive
10	VisionBeads	TCB-VB001-1	1.2mL	4°C	Light sensitive
11	Vision Live Suspension Solution	TCR-LSS001-500	500 mL	4°C	Handle under aseptic conditions. Avoid repeated freeze, thaw cycles.

* RT-Room Temperature   ** NA-Not applicable

III. REAGENT PREPARATION

VS Sheath is a 20X concentrated buffer solution. Each bottle provides sufficient concentrate to prepare **5L** of 1X Sheath fluid. To prepare **1L** of working solution (1X), add **50 mL** of 20X Sheath fluid to **950 mL** of Milli-Q/DI water.

IV. DESCRIPTIONS/INSTRUCTIONS FOR USE

1. VS Sort Cartridge

The VS Sort Cartridge is a microfluidics cartridge specially designed for the VisionSort platform, optimized to precisely channel individual cells or particles from the sample tube to the light interrogation and sorting points for both standard fluorescence flow cytometry and Ghost Cytometry applications. Integrated with VisionSort's gentle microfluidics system, the VS Sort Cartridge achieves rapid and high purity recovery of target cells with minimal damage during sorting. The VS Sort Cartridge is user-friendly, easy to install, and disposable, ensuring contamination free workflows.

To install the VS Sort Cartridge, tear open the aluminum foil and remove the cartridge from the plastic protective casing. Hold the cartridge by the cartridge region, at the edge of the cartridge, and avoid touching the glass area (near the center of the cartridge) which holds the microfluidic channels and sorting mechanism. Insert the cartridge into the VisonSort instrument and continue with the cartridge set up as described in the User Manual.
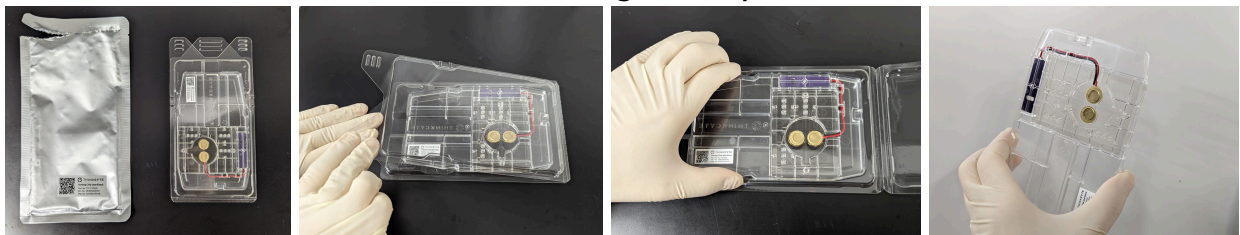
Note: VS Sort Cartridge may be used for 1 day. Reusing a cartridge over multiple days is not recommended and may lead to compromised sorting.
For More Information Refer to VS Sort Cartridge Datasheet

2. VS Wash Cartridge

The VS Wash Cartridge is designed to deliver VS Clean to VisionSort as part of routine cleaning, typically performed at the end of an instrument run cycle. During instrument start-up, VS Sheath is run through the VS Wash Cartridge to prepare the instrument for sample runs. The VS Wash Cartridge does not require cartridge calibration, but can be used to calibrate instrument fluidics and prevent crystallization of sheath fluid within the instruments microfluidics system. The VS Wash Cartridge is easy to install and reusable, promoting longer instrument lifetimes and high-quality analysis and sorting.

To install the VS Wash Cartridge, follow the same procedure as the VS Sort Cartridge. Following the washing procedure (and when otherwise not in use), place and store the wash Cartridge back in its original plastic protective casing to prevent damage to the cartridge.

Note: We recommend changing the wash cartridge once every two weeks or after every 10 runs. Extended use of a wash cartridge may compromise the instrument wash procedure.

3. 15 mL Tubes

Tubes are equipped with caps that have a crosscut permeable filter, preventing airborne particulates from contaminating samples during run cycles. The 15 mL tubes may be used in both the sample inlet and outlet (collection) ports, depending on the sample volume and duration of the sort. If a sample run is expected to take less than an hour, use a 15 mL tube for collection.

4. 50 mL Tubes

Tubes are equipped with caps that have a crosscut permeable filter, preventing airborne particulates from contaminating samples during run cycles. The 50mL tubes are used exclusively in the outlet (collection) ports to recover sorted samples. If a sample run is expected to take more than an hour, use a 50 mL tube for collection.

5. VS Sheath

VS Sheath is the delivery medium used to optimize alignment of cells into a single-file stream in the microfluidics compartments of VisionSort under normal, controlled operating conditions. VS Sheath fluid is stored and refilled in a steel-lined sheath tank located in the fluidics cart.

We recommended always beginning VisionSort startup with a full tank. Check the sheath tank fluid level before startup and refill the sheath tank when necessary (maintain the reservoir at 2.5 to 3 L full). The sheath fluid consumption rate is approximately 0.2 L/8 hrs. If sheath fluid runs out, the system will automatically stop and all data acquisition will cease. Refiling the sheath tank in the middle of an experiment may result in introduction of air bubbles or require recalibration of the cartridge.

Note: Please make 1X Sheath about overnight beforehand and pour gently into the sheath tank to avoid air bubbles.

6. VS Suspension Solution

VS Suspension Solution is a protein free buffer solution optimized for diluting VisionSort calibration and performance beads.

● For VS C-Beads, dilute **10µL** of VS C-Beads with **500 µL** of VS Suspension Solution.
● For VS P-RBW Beads, dilute **20 µL** of VS P-RBW Beads with **500 µL** of VS Suspension Solution.
● For Vision Beads, dilute **120 µL** of Vision Beads with **120 µL** of VS Suspension Solution.

7. VS Clean

VS Clean is an alkaline-based cleansing solution that helps remove nucleic acids and cellular debris from the instrument. VS Clean is also used to remove clogs during routine instrument cleaning cycles.

Apply **5 mL** of VS Clean solution for each of the two sample inlets for cleaning at the end of each day.

Note: Do not run VS Clean through a VS Sort Cartridge.
Warning: While using VS Clean, it is highly recommended to wear protective gloves and gear to avoid skin irritation, and be cautious not to let it come into contact with clothes as it may cause discoloration or damage.

8. VS C-Beads

VS C (Calibration)- Beads are used for calibrating the sample stream to the optical plane of focus and to check instrument stability during start-up procedures, ensuring reliable and reproducible data generation during instrument use.
Note: Beads should be stored at 4°C. Resuspend the particles by vortexing before use.

To set up automatic calibration
Fill the sample tube with **10 μL** of VS C-Beads and **500 μL** of VS Suspension Solution and mix well, then insert the tube into the tube holder. Place the tube holder into a sample inlet and close the door fully.

9. VS P-RBW Beads

VS P-RBW (Performance-Rainbow) beads contain a mixture of fluorescent particles of 6 different intensities with excitation wavelengths between 365-650 nm.

Note: Beads should be stored at 4°C. Resuspend the particles by vortexing before use.

To use VS P-RBW Beads
Fill the sample tube with **20 μL** of VS P-RBW Beads and **500 μL** of VS Suspension Solution and mix well, then insert the tube into the tube holder. Place the tube holder into a sample inlet and close the door fully.

10. VisionBeads

VisionBeads serve as an essential control to evaluate the performance of the VisionSort™ instrument in morphometric analysis. It is recommended to run Vision Beads daily to assess the classification performance of supervised machine learning (SVM) and the appearance of UMAP plots.

To use VisionBeads
Vortex the bottle to ensure even distribution of the beads.
Add **120 μL** of VS Suspension Solution to the FACS tube then add **120 μL** of the VS suspension solution to it . Vortex the sample before acquiring the beads on VisonSort.

For VisionBeads data analysis, please refer to the VisionBeads User Guide.

11. Vision Live Suspension Solution

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Vision Live Suspension Solution is specially formulated for optimal recovery of sorted fractions of live or fixed cell suspensions using VisionSort. It is a ready-to-use sterile buffered solution containing FBS as a protein carrier to minimize heterophilic antibody interference and sodium azide (0.09%) as preservative. The solution can be used as a diluent for Cell Staining, Cell Washing, Cell Suspension, or antibody preparations. In addition, the solution can also be used to suspend cells for sorting experiments.

To optimize viability and recovery during live cell sorting:
Sheath fluid alone does not contain the necessary materials to support cell needs for a prolonged time. Therefore, we recommend using cell culture media in the collection tubes. For optimal results in sorting live cells, add 8 mL of the mixture for every hour of sorting. When using a 50 mL tube, the maximum continuous sort is 3 hours (initial liquid volume of 24 mL, consisting of 12 mL each of Vision Live Suspension Solution and cell culture media).

FOR MORE INFORMATION VISIT https://thinkcyte.com/visionsort/

SDS AVAILABLE ON REQUEST AT https://thinkcyte.com/contact/

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