

Protocol Overview

VistaPlex™ Assay Kits for CellScape™

This overview highlights the required materials and steps to perform cell staining and imaging using VistaPlex Assay Kits on the CellScape precise spatial multiplexing platform, featuring ChipCytometry™ technology. Detailed protocols for individual assay kits are available with kit purchase.

REQUIRED MATERIALS & REAGENTS

Materials available from Canopy Biosciences

Item	Size	Catalog Number	Contents
<input type="checkbox"/> VistaPlex Assay Kit	10 reactions	AbKT-XXXX-10RXN	Antibody Diluent (50 mL) Primary Antibodies (100 µL each)
<input type="checkbox"/> CellScape Cell or Tissue Chip Kit	10 chips	PRSM-CHP-XXXX-010	10 CellScape Cell or Tissue Chips Fixation Buffer (60 mL) Wash Buffer (60 mL) Storage Buffer (50 mL) Chip Storage Box
<input type="checkbox"/> Wash Buffer	500 mL	PRSM-BUF-WASH-500mL	500 mL Note: Some kits require more than one 500 mL bottle of wash buffer.

General lab supplies

- CellScape platform for precise spatial multiplexing, including instrument, fluidics unit, and computer
- 2 mL amber microcentrifuge tubes (Eppendorf, Cat. # 022363379)
- Pipettes
- Pipette tips

CONSIDERATIONS BEFORE YOU BEGIN

- For research use only. Not for use in diagnostic procedures.
- Please consult the assay kit Safety Data Sheet for information regarding hazards and safe handling practices.
- Store VistaPlex Assay Kits at 4 °C and protected from light for long term stability.
- Antibody mixes remain stable at room temperature for the duration of assay execution on CellScape.
- Some antibodies can aggregate over extended storage times, which can be seen as bright spots in images. Filter antibody stocks with a 0.1 µm low protein binding syringe filter if aggregates are observed.



GENERAL WORKFLOW

1. Prepare sample: Isolate PBMCs or complete or tissue sectioning and antigen retrieval. Assemble sample into CellScape Chip.
2. Qualify sample: Initialize software programs and complete background scans.
3. Add antibodies and cycles: Load information about antibody identities and concentrations into software.
4. Order staining cycles: Set staining, imaging, and photobleaching parameters.
5. Prepare antibody working solutions: Following Staining Plan (see below example), create one antibody solution for each staining cycle.
6. Execute scans: CellScape automatically completes cycles of staining, washing, imaging, and signal removal.
7. Store chip: Flush with Storage Buffer, then sample can be stored on chip at 4 °C for future re-interrogation.
8. Export images: OME-TIFF files are exported for analysis on any compatible platform.

Example Staining Plan for Tissue Assay Kit. For each cycle, add 50 µL of each antibody to Antibody Diluent to generate antibody working solutions with a total volume of 500 µL. Indicated volumes are sufficient for staining of one Tissue Chip.

Cycle	Cap Color	Diluent Volume (µl)	Antibody Target	Antibody Volume (µl)	Canopy Clone	Label	Filter Set
1	Red	300	Biomarker A	50	AbKTXXXX-01	PE	FS560
			Biomarker B	50	AbKTXXXX-02	FITC	FS488
			Biomarker C	50	AbKTXXXX-03	PerCP-Cy5.5	FSPerCP
			Biomarker D	50	AbKTXXXX-04	BV421	FS421
			Biomarker E	50	AbKTXXXX-05	BUV395	FS395
3	Orange	350	Biomarker F	50	AbKTXXXX-06	PE	FS560
			Biomarker G	50	AbKTXXXX-07	FITC	FS488
			Biomarker H	50	AbKTXXXX-08	PE	FS560
4	Yellow	400	Biomarker I	50	AbKTXXXX-09	FITC	FS488
			Biomarker J	50	AbKTXXXX-10	PerCP-Cy5.5	FSPerCP
5	Green	350	Biomarker K	50	AbKTXXXX-11	PE	FS560
			Biomarker L	50	AbKTXXXX-12	FITC	FS488
			Biomarker M	50	AbKTXXXX-13	PE	FS560
6	Blue	400	Biomarker N	50	AbKTXXXX-14	FITC	FS488

Note: Tube cap color indicates the cycle in which the antibody is used.

For additional questions or technical support, contact support.canopy@bruker.com
 To learn more, visit canopybiosciences.com

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