

SONY



FX500 Exchangeable Fluidics Cell Sorter

Sony Biotechnology Inc.

FX500 Cell Sorter

Sorting Made Simple™

The FX500 is a microfluidics chip based cell sorter featuring a fully replaceable fluidics system. This lets researchers easily replace the entire fluidics path to control sample to sample carryover. The FX500 features comprehensive fluidics controls, advanced automation and easy to use software.

Patented CoreFinder™ technology automates instrument setup and streamlines workflow. The optical design offers up to three collinear excitation lasers - 488 nm, 561 nm and 638 nm and six fluorescence detectors. The six free-form PMTs enable detection of fluorescence signals from any laser based on filter setting.

To support true ease of operations and save time, the software guides users through acquisition, sort control, sort monitoring and analysis. An experiment-centric approach makes the software easy to learn and use. Data can be easily exported as FCS 3.0 or 3.1 format for use with 3rd party analysis software.

Options include a Sort Deposition System and Biosafety cabinet. The Sort Deposition System enables sorting and precise deposition into 96 and 384 well plates and other plate formats. The BCC300 class II biosafety cabinet has been validated by the Baker Company to meet National Sanitation Foundation 49 Standard (NSF49), European Standard 12469 and other standards for product and personnel protection.



- **Features replaceable fluidics system to minimize sample to sample carryover and streamline cell processing workflow**
- **Provides high level of automation enabling setup, sort calibration and monitoring with a push of a button**
- **Offers a range of microfluidics sorting chips (70 µm, 100 µm and 130 µm) permit sorting of a wide range of cell sizes**
- **Custom Class II Biosafety Cabinet option meets industry standards for personnel and product protection**

Innovative Fluidics

The FX500 features a unique replaceable fluidics system which includes an E-beam irradiated sorting chip and PEEK sample line as well as a gamma irradiated sheath line assembly. These single use consumables can be easily exchanged between experimental runs to control the sample to sample carryover.



E-beam irradiated sorting chip, PEEK sample line and gamma irradiated sheath line can be easily exchanged between experiments to control sample to sample carryover.

Sample line

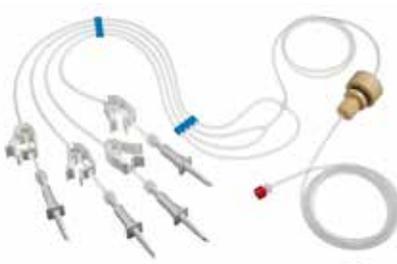
The sample is introduced into the E-beam irradiated sorting chip via the PEEK sample line. Exchanging these single use consumables between experiments controls sample to sample carryover.



E-beam irradiated PEEK sample line.

Sheath line assembly

The sheath line assembly is made of gamma-irradiated sheath line that can be linked to multiple sheath bags and connected to the chip via the chip connector.



Gamma irradiated sheath line assembly.

The absence of fluidics pumps and inline valves in this design allows for an easy replacement of the sheath line as needed.



Chip connector.

Sheath tank

A unique pressurized stainless sheath tank encloses a removable rack where sheath bags can be mounted. The sheath fluid from all the bags is channeled via a single sheath line to the chip.

Stainless sheath tank with removable rack for sheath bags.



Microfluidics Sorting Chip

The E beam irradiated sorting chip acts as an integrated flow cell-nozzle and can be easily installed and removed.

The sample and sheath fluid are injected into the chip micro-fluidic channels via inlet ports. Excitation lasers interrogate the sample within the chip before it passes through and is sorted as droplets. The chip is directly connected to the waste line and can be declogged via software options.

Unlike traditional systems that require nozzles to be removed for manual cleaning, the FX500 system uses an automated purge and prime cycle triggered by the software to clear the chip. This allows for continuous use without removing the chip. The chip is available in 70 μ m, 100 μ m and 130 μ m sizes allowing researchers to easily adapt the system to cell sizes.



Three sizes of sorting chips are available including 70 μ m, 100 μ m and 130 μ m sizes.

Optional Biosafety Solution

The BCC300 class A2 Level II biosafety cabinet is available as an option to provide both product and personnel protection. The cabinet measures 1,180 mm (W) x 991 mm (D) x 2,239 mm (H). Researchers can choose from BSC models which have integrated aerosol management system to ensure redundancy in operator protection.

Biosafety Standards Compliance

The cabinet was tested by the Baker Company with the FX500 sorter inside the work area. The testing validated that the biosafety cabinet with FX500 inside meets National Sanitation Foundation 49 Standard (NSF49), the European Standard 12469 and other international standards.

Built-in Aerosol Management

The cabinet incorporates a built-in aerosol management system which operates independently to actively evacuate aerosols from the sort collection chamber. The dual routes of aerosol evacuation provides additional operator protection.



Automation from Set-up to Analysis

Patented CoreFinder™ technology along with software and sensors provide automation across the workflow from set up to shut down to ensure consistency, save time and improve the accuracy of results.

Chip Loading & Positioning

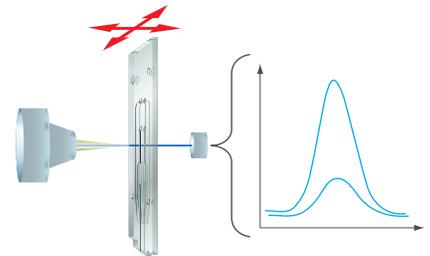
System set-up begins by installing the sorting chip on the chip loader. Once the chip is loaded, the sheath, sample and vacuum lines are automatically connected and sealed to their respective ports.



Easy to load sorting chip.

Automated Optical Axis Adjustment

The chip is automatically aligned to the lasers and optimized during setup using the Sony patented Blu-Ray™ technology for aligning and tracking laser position. On a daily basis, using AutoSetup beads, the X and Z position of the chip is adjusted to ensure consistent results.

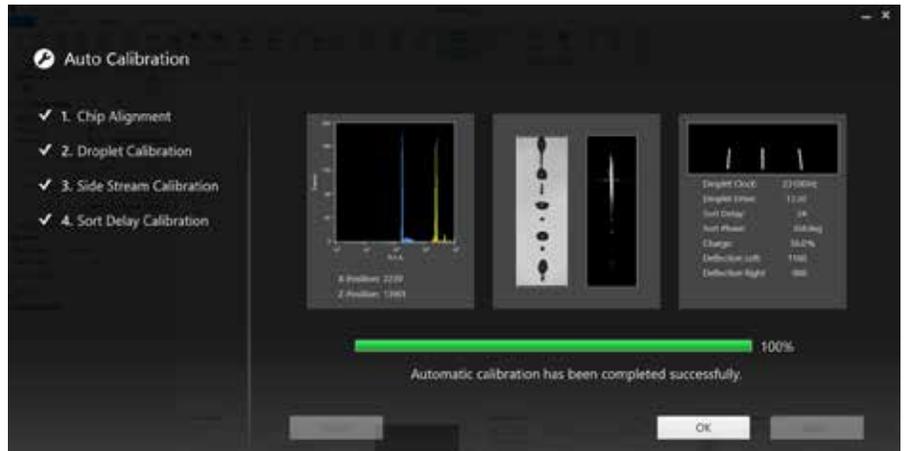


Automation for Droplet Calibration, Side Stream Calibration and Drop Delay Calibration

The droplets are automatically calibrated by adjusting the frequency and the drop drive to achieve optimal break off point (BOP) for each type of sorting chip.

Automated side stream calibration is achieved by calculating the angle and the position of the side streams and making adjustments for tube and plate sorting during setup. This ensures that the sort stream is centered in the collection tube automatically without manual adjustment.

Drop delay calibration is accomplished using a dedicated laser and camera that perform automatic, real-time analysis of binary images of droplet containing AutoSetup beads. A patented algorithm calculates optimal drop delay by computing the relationship between bead positions and drop delay.



Easy to use software automates sort set up.

Automated Sort Monitoring

The software actively monitors and makes adjustments to the drop drive to maintain a stable breakoff point. This allows consistently good sort performance and clog detection to facilitate walk-away operation.



Software

A ribbon interface logically organizes features to make them rapidly accessible. An experiment-centric approach makes the software easy to teach, learn and use. The software is intuitive and supports automated setup to acquisition and analysis. The software generates FCS 3.0 and FCS 3.1 files that also can be exported to third party analysis tools.

System Startup

Upon start up, the system initiation includes diagnostics that ensure all subsystems are properly working. Once verified, system status and green ready message are displayed on the LCD monitor on the front of the instrument. The LCD monitor on the front of the instrument displays status information during operation.



The LCD monitor on the front of the FX500 displays status information during operation.

Alignment and Calibration

The system automatically and precisely aligns the sorting chip to the lasers and calibrates the droplets, side streams and drop delay using autoseed beads.



Sort deposition unit.

A ribbon interface logically organizes features to make them rapidly accessible. An experiment-centric approach makes the software easy to teach, learn and use. The software is intuitive and supports automated setup to acquisition and analysis. The software generates FCS 3.0 and FCS 3.1 files that also can be exported to third party analysis tools.

Software wizards include step-wise workflows that guide users through start up, multicolor compensation, sorting and system shutdown.

Creating Experiments

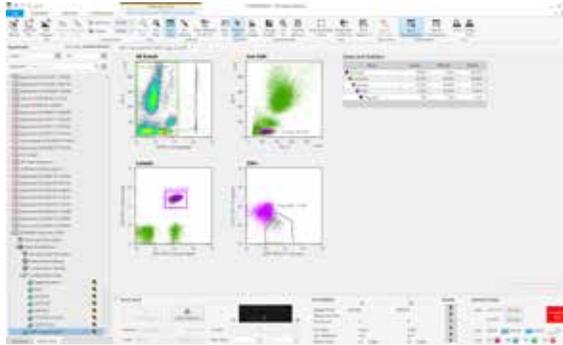
Users can create an experiment by using templates from a selection of recent experiments. When a new template is created, guided dialogs prompt the user to choose experiment settings which contains sample groups, tubes and pulse parameters for data acquisition. Prior to starting acquisition a user can setup up compensation tubes.



Choosing a template or a recent experiment on the left of the window displays the experiment structure on the right.

Data Analysis and Display

Data is displayed as dot plots and histograms on worksheets and populations can be marked using gates. The software has a number of tools to select, adjust, label and measure statistics of target populations. Data can be analyzed within the software or exported as FCS 3.0 or 3.1 formats to use with third party analysis software.



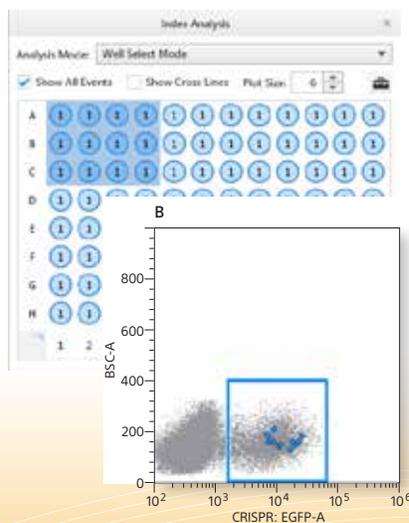
The Experiments tab provides easy access to all lists of previous experiments as well as graphics tools for visualization and statistics tools for analysis.

Sorting

Once events are gated on plots, targeted cells can be sorted for further analysis. The software offers different sort modes to provide different levels of purity and yield as well as single cell sorting mode to support optimal results. All sort controls are managed within a simple dialog box.

Sort Deposition System and Index Sorting Software

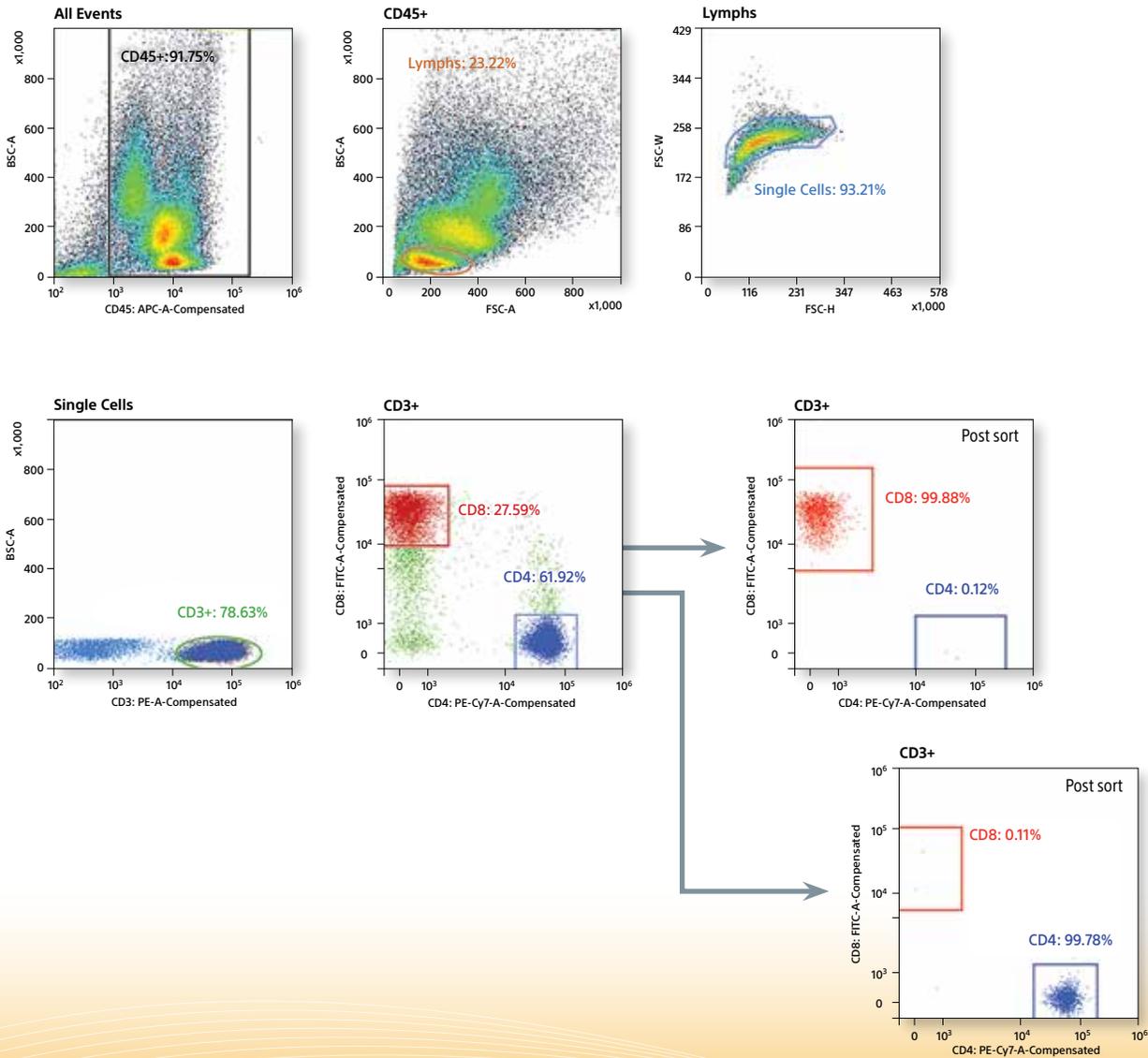
The FX500 supports sorting into tubes by default. The Sort Deposition System enables sorting and precise deposition into 96 and 384 well plates and other plate formats. Index sorting software records the X and Y coordinates of each event sorted into a multi-well device. This feature allows researchers to track the scatter and fluorescence intensity of individual cells sorted in each well.



High purity sorting of T cells

In this data, cells from buffy coat preparation from human blood were used at a final concentration of 20 million/ml. Cells were stained with 10 microliters of human anti-CD45 APC (Cat # 2120060), anti-CD3 PE (Cat # 2102040), anti-CD8 FITC (Cat #2323520) and anti-CD4 PECy7

(Cat #2102560) from Sony Biotechnology Inc. were used for staining the cells for 30 minutes. The stained sample was run on FX500 cell sorter using the 70um sorting chip. At an event rate of 20,000 events/s, CD8+ and CD4+ were sorted with a purity of >99% and efficiency of >75%.



Specifications

Filter Guide

	FL1	FL2	FL3	FL4	FL5	FL6
EGFP	●					
FITC	●					
Alexa Fluor® 488	●					
EYFP	●					
mCitrine	●					
CFSE	●					
PE		●				
PE-Texas Red®			●			
Propidium Iodide			●			
dsRed			●			
tdTomato			●			
mCherry			●			
mPlum				●		
7-AAD				●		
PE-Cy®5				●		
PerCP				●		
PE-Cy5.5					●	
PerCP-Cy5.5					●	
PE-Cy7						●
APC				●		
Cy5				●		
Alexa Fluor 647				●		
APC-Cy5.5					●	
Alexa Fluor 700					●	
APC-Cy7						●
APC-Alexa Fluor 750						●

Filter Set

LP1 639LP	FL1 525/50	FSC 488/17F 488/17F
LP2 600LP	FL2 585/30	
LP3 561LP	FL3 617/30	BSC 488/17B 488/17B
LP4 752LP	FL4 665/30	
LP5 685LP	FL5 720/60	
	FL6 785/60	

Specifications

Optics	Excitation lasers	488 nm, 638 nm and 561 nm laser
	Output power	15 mW (typical output) for each laser
	Beam alignment	Collinear optical system
	Detection parameters	8
	Analog-to-digital converters (ADC)	8-channel 20-bit, 100MHz
	Pulse measurement	Height, Area, Width
Fluidics	Sample tube	Single, auto-loading tube
	Tube types	0.5 ml, 1.5 ml, 5 ml and 15 ml
	Sort devices	2-way tube, multiwell plates, PCR tubes, slides
	Temperature control	5°C * and 37°C **
	Agitation unit	Eccentric rotation
	Magnetic drive	300 rpm speed
	Sorting chip size	70 µm and 100 µm, 130 µm (beta)
Sort Performance	Event rate	100,000 eps
	Sorting speed	Using the 70 µm sorting chip at 52 kHz an average threshold of 12,000 events per second can be achieved with purity >= 98% and yield >= 80% using the Normal Sort Mode. A higher threshold rate can be achieved with purity >= 98% with a decrease in yield based on Poisson's statistics.
	Scatter resolution	0.5 µm
	Fluorescence resolution	FITC, PE <= 2.5% Half-peak coefficient of variation (HPCV)
	Fluorescence sensitivity	MESF: FITC 120; PE 110 ***
	Linearity	FITC, PE : R2 <= 0.98 with 8 peak Spherotech beads
Ancillary	Dimensions	W: 21.7" (55 cm) x D: 21.7" (55 cm) x H: 28.4" (72 cm)
	Fluidics cart	W: 28.3" (71.5 cm) x 13.6 in (34.5 cm) x 27.8 in (70.5 cm)
	Weight	220.5 lb (100 kg)
	Fluidics cart	81.6 lb (37 kg) (Dry weight)
	LCD panel	7-inch, 800 x 480 pixels
	Power supply	100 V 50/60 Hz, 120 V 60 Hz
	Power consumption	500 W (max.)
	Operating temperature	17.5 to 27.5°C
	Relative humidity	20 to 80%
Compliance	Operating system	Microsoft® Windows® 10 Professional, 64 bit
	Data file structure	Flow Cytometry Standard (FCS) 3.0 or 3.1
	Safety standards compliance	UL, CE, CSA

* Typical for sample and collection tubes

** Typical for sample tube only

*** Calculated using Spherotech beads (RCP-30-5A) according to Sony's specifications

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