

Healthy Cells Broad Capabilities Better Science

Gentle, customizable benchtop microfluidic cell sorting.





High cell integrity without compromise •••

The WOLF was originally created by a team of scientists and engineers who wanted to solve a classic challenge in biological research: how to effectively and easily sort high-quality cells. Now, as science continues to move forward into increasingly complex realms, the WOLF is moving forward with it. The WOLF G2 instrument has significantly expanded the capabilities of gentle benchtop microfluidic cell sorting with two lasers and up to nine colors, while maintaining simple workflows for either bulk sorting or single-cell dispensing. Single-cell sorting can be completed in 96- or 384-well plates when using the WOLF G2 in conjunction with the N1 Single-Cell Dispenser. This flexibility in performance, along with the additional abilities of the second laser, makes it ideal for use in many different research fields and application areas like single-cell genomics, cell line development, gene editing, antibody discovery, immunology, infectious disease, basic research, and more.

WOLF G2 Cell Sorter



Healthy Cells

At < 2 psi, the WOLF G2 are gentler than any conventional cell sorters, enabling healthier cells post-sort, especially for engineered lines, primary cells, and stem cells.



High Sensitivity and Resolution

All laser configurations afford < 250 MESF sensitivity, along with forward and back scatter, providing as low as 1 µm resolution.



Contaminant- and Biohazard-Free

Disposable, aerosol-free microfluidic cartridge isolates the sample from the environment, while protecting the user from exposure.

	$\overline{2}$
	7

Compact

At under 2 cubic feet, NanoCellect's benchmark for access and performance allows every lab for the flexibility to do analysis and sorting into tubes or 96- and 384-well plates.



Simple and at your Bench

Intuitive software, fixed optics, no fluidics cart and less than one minute clean-up time.

Expanding the WOLF's Capabilities

With two lasers and up to nine fluorescent channels, the WOLF G2 aligns with a broad set of research applications and experiments. Three different laser configurations allow options specific to your needs.

Applications •••

Gene Editing



- Gentle microfluidic cell sorting with the WOLF G2 uses pressures of below 2 psi. The combined power of the WOLF G2 and the N1 Single-Cell Dispenser eliminates the main challenges typically facing pluripotent stem cell processing.
- Without decompression shock and shear stress, single-cell deposition results in high monoclonal outgrowth.
- This improved workflow will allow for higher throughput pluripotent stem cell research, which is valuable for both basic and clinical research.

Therapeutic

Antibody Production

Infectious Disease



Viral Particles or Antigens

Viral Culture

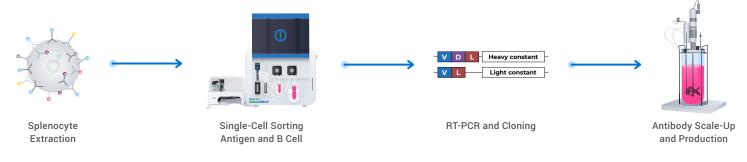
• The WOLF G2 microfluidic cell sorter is aerosol-free and compact enough to fit into standard biosafety hoods, making it ideal for infectious disease research.



Biohazard Antigen and B cell Sorting

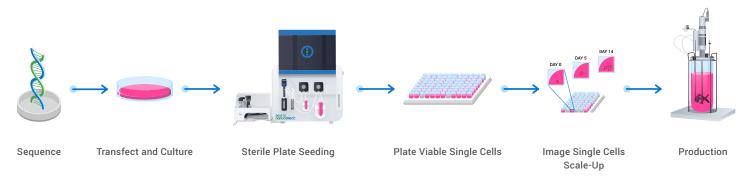
• The WOLF G2's disposable cartridges also avoid crosscontamination and create a safe and effective workflow for the kind of speed and efficiency required to combat infectious disease threats.

Immunology and Antibody Discovery



- The WOLF G2 Cell Sorter provides a simple cell sorting solution that gently sorts high antibody-producing clones and dispenses single cells for optimal clone outgrowth.
- With two lasers and up to nine fluorescent channels, the WOLF G2 provides the most comprehensive benchtop cell sorting solution.

Cell Line Development



- The WOLF G2 Cell Sorter protects cell viability after sorting and improves sorting efficiency by increasing the number of viable clones growing per plate.
- Contaminant- and biohazard-free, the WOLF G2 Cell Sorter's rapidly exchangeable cartridge and tubing set eliminates carryover between samples and allows for quick and easy cleanup.
- When paired with the N1 Single-Cell Dispenser, the WOLF G2 can select viable single cells and plate them into 96- or 384- well plates.
- Low pressure microfluidics greatly improves cell integrity over conventional droplet sorters, resulting in high-outgrowth and high-titer monoclonal lines.

Genomics

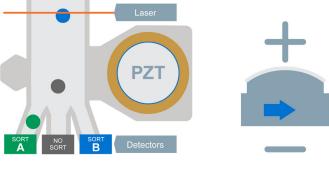


- The WOLF G2's gentle microfluidic system eliminates unintentional sequencing and analysis of dying or off-target cells or debris while maintaining cell integrity and avoiding stressing cells and affecting downstream data.
- Researchers can sort cells with a wide range of genome sizes, including human, plant, and animal cells or microorganisms like yeast and bacteria, providing insight on population genetics or disease.
- The WOLF G2 also allows labs to be completely flexible when it comes to sequencing research and eliminates carryover with a 100% disposable fluidic cartridge.

Microfluidic Cartridge Technology •••

The WOLF G2 Cell Sorter uses patented, microfluidic-based sorting with robust laser-excitation and sensitive PMT detectors to isolate mammalian cells, microbes, plant cells and more. A gentle and precise piezoelectric actuator directs cells into collection channels and allows analysis and sorting in a disposable format. This eliminates sample-to-sample contamination and biohazard exposure or cleanup.

- Unique to NanoCellect are our disposable cartridges that allow for bulk sorting or single-cell sorting.
- The sorting cartridges use a piezoacoustic actuator that gently directs cells into collection channels; an embedded cell sorting verification system gives instant feedback of sorting accuracy.
- This technology allows the WOLF G2 to sort up to 200 cells per second with high accuracy and effective recovery.
- Sort two selected cell populations with bulk sorting while the remainder of cells collects in a third channel.
- Deposit 1 to 100 cells per well in a 96- or 384 well plate using a single-cell sorting cartridge along with the N1 Single-Cell Dispenser accessory.



Magnified Sorting Junction

Actuator

Key Benefits



Sterile

Cartridges are individually-packaged and sterilized using ethylene oxide ensuring sterility for safe handling and storage.

Disposable

Anything that the sample or sheath fluid touches is disposable.



Gentle Sorting

A gentle sorting mechanism results in improved viability of cells and higher outgrowth.



No aerosols

Sorting happens inside the fully enclosed cartridge, preventing the creation of dangerous aerosols.



Easy clean-up

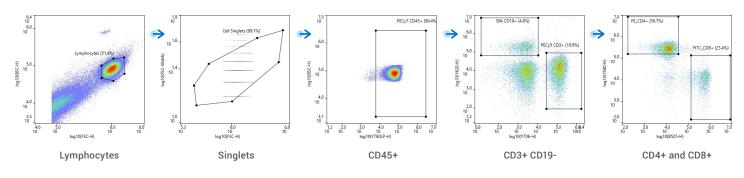
Clean-up in one minute and simply discard the cartridge.



WOLF G2 Cell Sorting •••

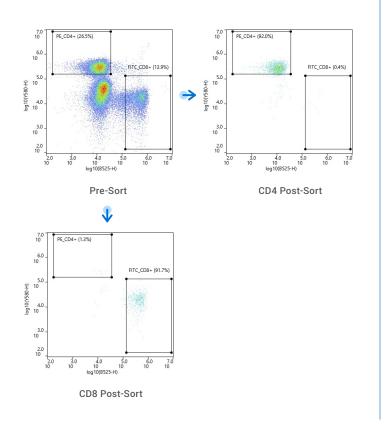
5-Color Immune Cell Sorting:

To verify sorting performance, CD4 and CD8 T Cells were sorted from BioLegend's PBMC Veri-Cells. They were gated from the CD3+CD19-CD45+ lymphocyte population.



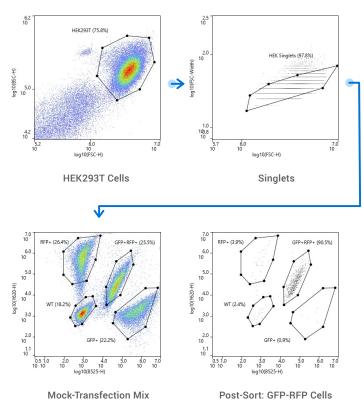
5-Color Sort Performance:

CD4 and CD8 T Cell populations were evaluated based on gates. CD4 T cells were enriched to 92.0% from a 26.5% target population. CD8 T cells were enriched to 91.7% from a 13.9% target population.



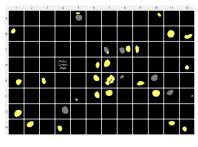
Transfected Cell Lines:

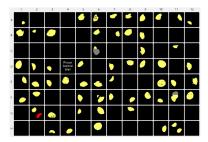
Four HEK293T cell lines were mixed to mimic a GFP+RFP+ dual-expressor transfection. To verify sorting performance, the GFP+RFP+ cells were sorted from the rest to result in 90.5% post-sort purity.



Monoclonal HEK Outgrowth

The same 25% GFP+RFP+ dual-expressor cell mix was also sorted for single cells into 96-well plates and incubated for 14 days. As a rigorous limiting dilution comparison, 100% GFP+RFP+ dual-expressor cells were dispensed at 1 cell/well. The WOLF G2 with N1 plate still yielded more than a 2-fold increase in targeted monoclonal colonies.





N1 Single-Cell Dispenser: 1 cell/well from

25% GFP+RFP+ HEK293T cell mix

Limiting Dilution: 1 cell/well from 100% GFP+RFP+ HEK293T cell mix

Little to no fluorescence

RFP+ only fluorescence

GFP+RFP+ fluorescence

N1 Single-Cell Dispenser

Designed to sort and dispense into 96- and 384well plates, the N1 provides higher rates of singlet detection compared to cell printers or limiting dilution. Users can perform simple, label-free dispensing or advanced multicolor panel single-cell dispensing.

Plate sorting specifications

Time to plate (96 wells): 3 - 8 minutes Time to plate (384 wells): 32 minutes Droplet volume: $3 - 10 \ \mu L$ Sample plate options: 96 or 384 wells (flat bottom, V-bottom, U-bottom, PCR)



WOLFViewer Software

The WOLFViewer software has an intuitive workflow menu that walks users through their experimental process, and is designed for both novice and expert users. New users can be performing their first sorts in about 20 minutes and can be on their own on day one.

Plate sorting specifications

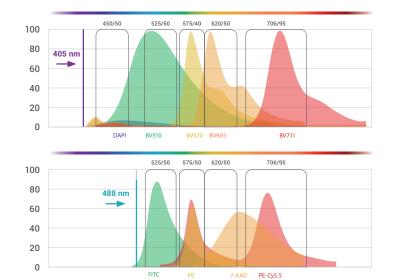
System power up: 3 minutes System setup and calibration: 20 minutes System shutdown: 3 minutes Additional features: Auto-alignment, intuitive compensation, advanced coloring and gating options, FSC files compatible with FlowJo

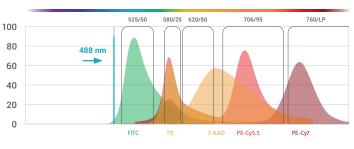


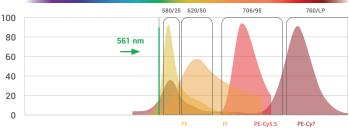
WOLF G2 Configuration Guide •••

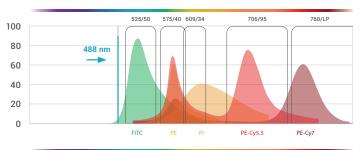
The WOLF G2's three possible laser configurations significantly enhance the number of fluorescent markers that may be utilized.

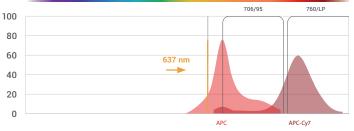
	WOLF G2 405 nm
Excitation Source	Emission Detection: Filters
405 nm, 55 mW DPSS laser	 450/50 (DAPI, BV421*) 525/50 (BV510*) 575/40 (BV570*) 620/50 (BV605*) 706/95 (BV711*)
	WOLF G2 488 nm
Excitation Source	Emission Detection: Filters
488 nm, 55 mW DPSS laser	 525/50 (FITC, GFP) 575/40 (PE) 620/50 (PI)











		WOLF G2 488 n	m
88	Excitation Sc	urce Emissior	Detection: Filters
WOLF G2 488	488 nm, 55 m DPSS laser	 580/2 620/50 706/9 	
		WOLF G2 561 ni	n
Excit	ation Source		ion: Bandpass Filters
	nm, 55 mW Slaser	 580/25 (PE) 620/50 (PI, n) 706/95 (7-A4) 	57

- 706/95 (7-AAD, PE-Cy5.5[®])
 700 D (DE 0.7[®])
- 760LP (PE-Cy7®)

	WOLF G2 488 nm
Excitation Source	Emission Detection: Bandpass Filters
488 nm, 55 mW DPSS laser	 525/50 (FITC, GFP) 575/40 (PE) 609/34 (PI) 706/95 (PE-Cy5.5[®]) 760LP (PE-Cy7[®])
	WOLF G2 637 nm
Excitation Source	Emission Detection: Bandpass Filters
637 nm, 55 mW DPSS laser	 706/95 (APC) 760LP (APC-Cy7[®])

WOLF G2 488/637

WOLF G2 488/561

WOLF G2 405/488

Fluorophore Guide •••

Instrument	Exci	tation Laser	Emission Filter	Fluorescent Dyes	Fluorescent Proteins
405 nm			450/50	Alexa Fluor® 405, DAPI, Brilliant Violet™ 421	eBFP, Cerulean
		525/50	Pacific Green, Brilliant Violet™ 510	AmCyan, CFP	
	405 nm	575/40	Pacific Orange, Brilliant Violet™ 570, Qdot® 565, Qdot® 585		
5/48	WOLF G2 405/488	620/50	Brilliant Violet [™] 605, Qdot® 605, Qdot® 625, 7-AAD		
2 40			706/95	Qdot® 705, Brilliant Violet™650, Brilliant Violet™711	
ILF G			525/50	Alexa Fluor [®] 488, FITC, SYTOX [®] Green	eGFP, eYFP, mCitrine
MO			575/40	PE	
		488 nm	620/50	PE-Texas Red®, PE-Alexa Fluor® 594, ECD, PE/Dazzle™ 594, 7-AAD	
			706/95	PE-Cy5 [®] , PE-Cy5.5 [®] , PerCP-Cy5.5 [®]	
			525/50	Alexa Fluor® 488, FITC	eGFP, eYFP, mCitrine
3/561 WOLF G2 488		580/25	PE	mKate, mBeRFP, CTOFP1, DsRED	
	9 488 nm 100	488 nm	620/50	PE-Texas Red®, PE-Alexa Fluor® 594, ECD, PE/Dazzle™ 594, 7-AAD	
488,			706/95	PE-Cy5®, PE-Cy5.5®	
WOLF G2 488/561		760LP	PE-Cy7®, PE-Vio®770		
NOL			580/25	PE	DsRED, tdTomato
	561 nm	561 nm	620/50	Texas Red®, PE-Texas Red®, Alexa Fluor® 594, PE-Alexa Fluor® 594, ECD, PE/Dazzle™ 594, 7-AAD	mCherry, mStrawberry
			706/95	PE-Cy5®, PE-Cy5.5®, PerCP, 7-AAD, DRAQ5, DRAQ7	
		760LP	PE-Cy7®, PE-Vio®770, ", DRAQ5™, DRAQ7™		
WOLF G2 488/637			525/50	Alexa Fluor® 488, FITC	eGFP, eYFP, mCitrine
		488 nm	575/40	PE, PE-610	eYFP, mCitrine
			609/34	PI	
			706/95	PE-Cy5®, PE-Cy5.5®	
10LF			760LP	PE-Cy7®, PE-Vio®770	
3		637 nm	706/95	APC, Alexa Fluor® 633	
			760LP	APC-Cy7®, APC-Horizon™7	

Cy™ is a trademark of GE Healthcare.

Alexa Flour®, Texas Red®, SYTOX®, and Qdot® are registered trademarks of Life Technologies Corporation.

DRAQ5[™] and DRAQ7[™] are trademarks from Biostatus Limited.

Brilliant Violet[™] is a trademark of Sirigen Group Ltd.

 $\mathsf{Vio}^{\circledast}$ is a registered trademark of Miltenyi Biotec GmbH.

Dazzle™ is a trademark of BioLegend.

For more information on compatible fluorophores, visit nanocellect.com or email info@nanocellect.com

WOLF G2 Specifications •••

Fluidics			
Sample input	1.5, 2.0, and 5.0 mL tubes		
Sheath input	50 mL conical tubes		
Sheath fluid	PBS or buffer of choice		
Sheath fluid usage	9.6 mL/hour		
Sample flow rate	24 µL/minute		
Sheath flow rate	160 μL/minute		
Sample line volume	50 μL		
Minimum sample volume	100 μL		
Tubing diameter (inner)	200 to 500 µm		
Flow cell	200 x 70 µm		
Smaller channel diameter	70 µm		
Sample pressure	< 2 psi		
Sample output (bulk sorting)	1.5 mL or 5 mL tubes		
Sample output (single cell)	96- or 384-well plates (flat/U/V bottom or PCR)		

Performance			
Scatter sensitivity	< 1.5 µm by FSC or BSC		
Scatter resolution	Resolves lymphocytes, monocytes, and granulocytes		
Fluorescence sensitivity	< 200 MESF FITC (using 488 nm laser) < 250 MESF PE (using 561 nm laser)		
Fluorescence resolution	9-peak separation with SPHERO™ Rainbow Calibration Particles		
Analysis speed	2,000 events/second		
Sorting	1- and 2-way		
Back-to-back sorting speed	200 events/second		
Absolute counts	Yes		
Volumetric counts	Yes		
Warm-up time	Less than 1 minute		
Sorting purity	Up to 99% purity		

N1 Plate sorting specifications			
Time to plate (96 wells)	3 - 8 minutes		
Time to plate (384 wells)	32 minutes		
Droplet volume	3 - 10 µL		
Sample plate options	96 or 384 well (flat bottom, V-bottom, U-bottom, PCR)		

	Optics
Laser Profile	20 x 90 µm
Scatter detection	Forward (0 degrees, +/- 15) Back (180 degrees, +/- 15)
Excitation & emission detection	See WOLF G2 Configuration Guide
Optical alignment	Fixed alignment, no maintenance required

Instrument specifications		
WOLF G2 Dimensions	14.8H x 18.0W x 13.6D inch (37.6H x 45.8W x 34.5D cm)	
WOLF G2 Weight	54 lbs / 24.5 kg	
WOLF G2 Electrical	AC Input: 100-240V, 50-60Hz, 2A	
N1 Dimensions	8.42W x 6.5H x 8.34D in (21.4 x 16.5 x 20.9)	
N1 Weight	5.5 lbs / 2.5 kg	
N1 Electrical	DC Input: 24V, 1A	

 ${\sf SPHERO}^{\tiny ™}$ is a trademark of Spherotech, Inc.

NanoCellect Technical Support •••



Onboarding

NanoCellect is committed to provide you with the highest level of support and expertise for your cell sorting workflow. We are dedicated to delivering the very best solutions and assistance to help you improve the quality of your research.



Training

Our Sales and FAS teams will assist you during initial instrument installation and for additional training options. Online training videos and other material are always available and being updated on our website's dedicated Knowledge Base.



Technical Support

Our expert technical support, including dedicated Field Application Scientists and Customer Success teams respond quickly and are available for you when you need them. Additional support options include remote TeamViewer sessions, in-person training and repairs, and online educational resources.



Service Options

We now offer Installation and Operational Qualification services to verify and document that your WOLF system and accessories are installed, operating, and performing according to our specifications. Additionally, our two Service Plan options allow you to pick the level that best fits the needs for your budget and lab and include access to NanoCellect technical support, WOLFViewer software updates, preventive maintenance, replacement parts, and site visit labor and expenses.

For more information, visit nanocellect.com or email info@nanocellect.com





Class 1 Laser Product Designed and Built in San Diego, California

9525 Towne Centre Dr., Suite 150 San Diego, CA 92121 (877) 745-7678

