

invitrogen



A counter that thinks like a cell biologist

Countess 3 Automated Cell Counters
Accuracy | Precision | Time savings | Convenience

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Countess 3 Automated Cell Counters

Artificial intelligence takes accuracy to a higher level

Thermo Fisher Scientific offers two high-performance cell counters designed to meet the needs of any lab. The Invitrogen™ Countess™ 3 and Countess™ 3 FL Automated Cell Counters utilize artificial intelligence, applying a deep-learning neural network algorithm to generate highly accurate cell counts even for challenging cell sample types. The machine-learning algorithm was taught to the systems by our scientists to help ensure accuracy and precise cell counting, and avoid the user variation associated with manual hemocytometer cell counting.

The Countess 3 Automated Cell Counters come equipped with automated features like autolighting, autofocus, and autosave to provide convenience and time savings.

- **Accuracy**—highly accurate machine-learning algorithm applied to cell counting
- **Precision**—auto-lighting and autofocus minimize user inputs and variability
- **Time savings**—rapid capture and autosave features help generate and save results fast
- **Convenience**—built-in pre-dilution and cell splitting calculators, histograms and gating, and a PDF report of results
- **Affordable**—both Countess 3 instrument models are compatible with the Invitrogen™ Countess™ Reusable Slide



Table 1. Features of Countess 3 Automated Cell Counters.

Features	Countess 3 Automated Cell Counter	Countess 3 FL Automated Cell Counter
Machine-learning algorithm	✓*	✓*
1,280 x 800 pixel LCD screen	✓*	✓*
Wi-Fi-enabled cloud connectivity	✓*	✓*
Brightfield image and count	✓	✓
Brightfield autolighting	✓	✓
Brightfield rapid capture	✓*	✓*
Fluorescence images and counts	N/A	Two interchangeable fluorescence channels (user's choice)
Fluorescence autolighting	N/A	✓*
Saturated pixel indicator	N/A	✓*
Autofocus	✓	✓
Autosave	✓*	✓*
Countess chamber counting slides	✓	✓
Countess reusable slide	✓*	✓
Pre-dilution calculator	✓*	✓*
Cell splitting calculator	✓	✓
Single-cell data	✓*	✓*

* New features of the Countess 3 Automated Cell Counters.

Features of the Countess 3 instruments

Accuracy and precision

Countess 3 Automated Cell Counters utilize machine-learning algorithms to provide fast and accurate focus determination as well as cell and viability counts. Countess instruments are compatible with a broad range of cell types and have been verified for use with several commonly used cell lines and primary cell types (Figure 1 and Table 4).

Upon insertion of the slide, the Countess instrument will automatically provide optimized illumination and focus on the cells, which helps to minimize the user variation associated with manual focusing and lighting adjustments. Cells ranging in size from $\sim 4\ \mu\text{m}$ to $60\ \mu\text{m}$ and cell suspension density ranging from 1×10^4 to 1×10^7 cells/mL are optimal for counting.

Accuracy of the Countess 3 Cell Counter compared to the Invitrogen™ Attune™ NxT Flow Cytometer and a manual hemocytometer

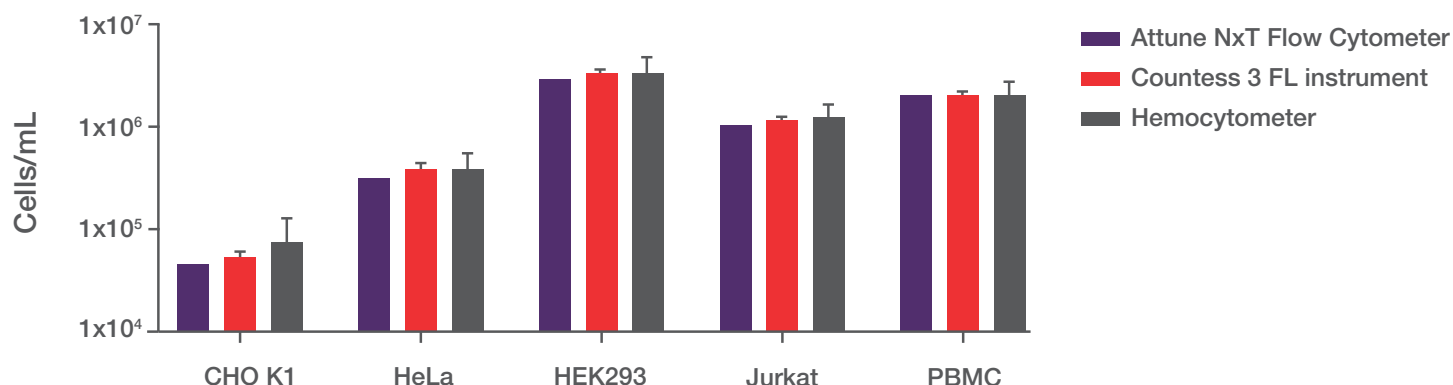


Figure 1. Machine-learning algorithms result in highly accurate cell counts, comparable with flow cytometer counts. CHO-K1, HeLa, HEK293, Jurkat, and human peripheral blood mononuclear cells (PBMCs) were counted using the Attune NxT Flow Cytometer (purple bar), a Countess 3 FL Automated Cell Counter (red bar), and manual counting using a hemocytometer and microscope (gray bar). The Countess 3 FL Automated Cell Counter and hemocytometer bars represent an average of 6 counts. Error bars indicate standard deviation from 6 independent counts.



Counting clumps, not debris

The machine-learning algorithm of the Countess 3 and Countess 3 FL Automated Cell Counters can clearly identify cell boundaries within clumps of cells and ignore debris, resulting in accurate cell counts (Figure 2).

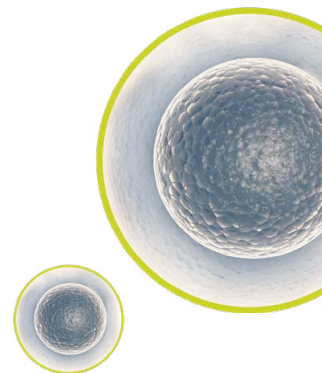
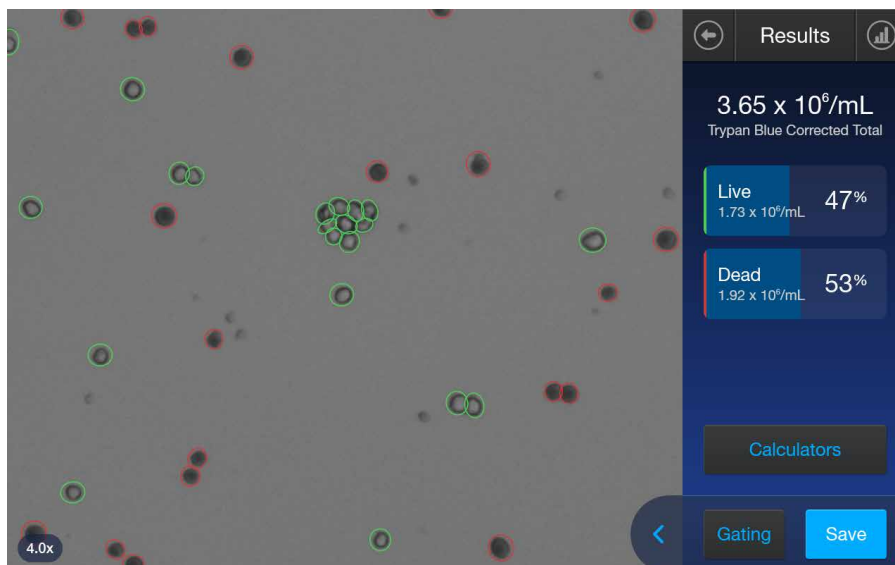


Figure 2. Machine-learning algorithms generate accurate counts with clumpy cells and sample debris. The Countess 3 FL Automated Cell Counter was used to count RAW cells, which are both small and have a propensity to clump. The counting algorithm can resolve the cells in the clump, and properly segment and count the cells. Debris is properly omitted from counts.

Counting PBMCs

Rodent PBMCs are small cells around 5–7 microns and can be challenging to count whether manually on a hemocytometer or with an automated cell counter. The machine-learning algorithm used by the Countess 3 Automated Cell Counters is robust and accurately counts these cells in both brightfield and fluorescence modes (Figure 3).

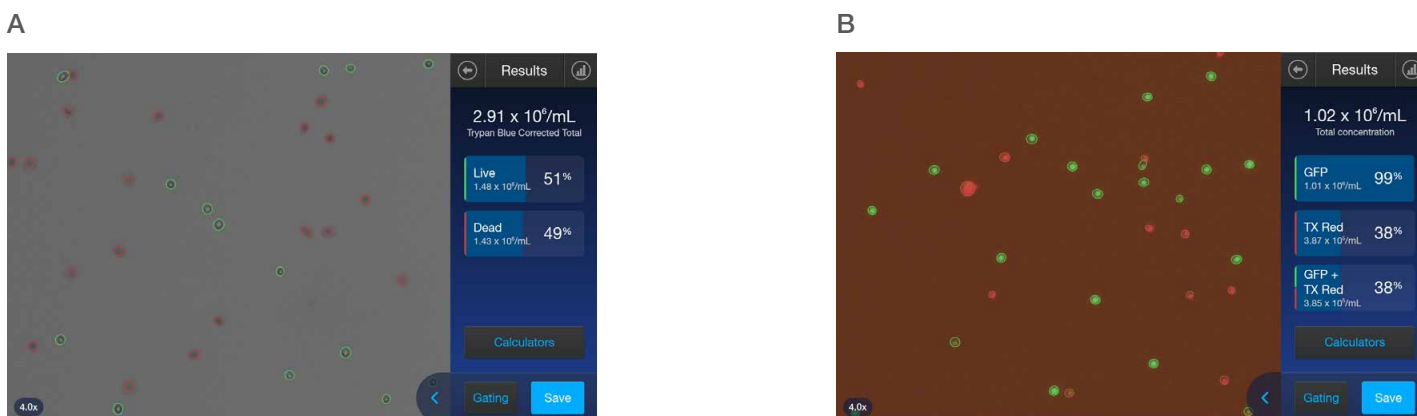


Figure 3. Rodent PBMCs accurately counted with machine-learning algorithm. The Countess 3 FL Automated Cell Counter was used to count small rodent PBMCs. Counts were performed with (A) brightfield lighting and (B) fluorescence lighting.

Time savings

The Countess 3 and Countess 3 FL Automated Cell Counters streamline the process of cell counting, eliminating many of the tedious steps associated with manual cell counting (Figure 4). This additional time can be applied to completing cell culture processing and other activities in the lab, resulting in a significant advantage when switching from manual to automated cell counting.

Comparison of cell counting processes

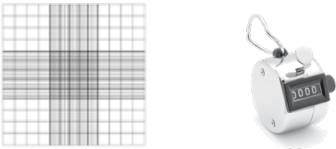
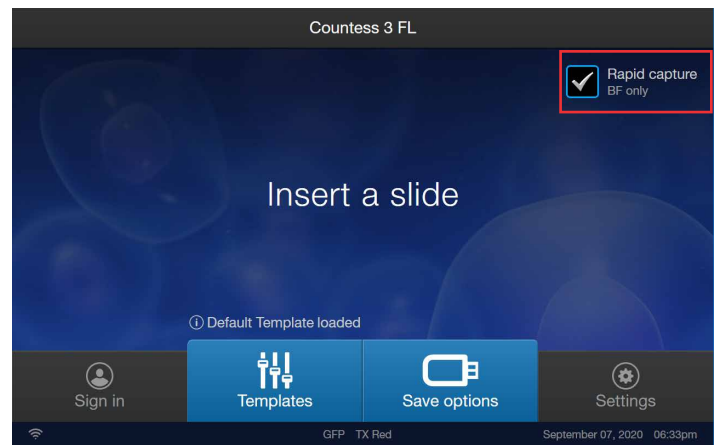
Manual counting with a hemocytometer and microscope	Countess 3 Automated Cell Counter with Countess Reusable Slide	Countess 3 Automated Cell Counter with Countess Cell Counting Chamber Slides
		
Counting 4 quadrants	- Using Rapid capture - Built-in autolighting and autofocus - Using Autosave	- Using Rapid capture - Built-in autolighting and autofocus - Using Autosave
- Clean & dry hemocytometer - Clean & dry coverslip - Load sample into hemocytometer - Mount on microscope - Adjust lighting - Adjust focus - Count cells - Write down counts - Calculate cell concentration - Calculate volumes for next steps - Clean hemocytometer - Clean coverslip	- Clean reusable slide - Clean coverslip - Load sample into reusable slide - Insert slide into Countess 3 counter - Use built-in calculator to calculate volumes for next steps - Clean reusable slide - Clean coverslip	- Load sample into chamber slide - Insert slide into Countess 3 counter - Use built-in calculator to calculate volumes for next steps
- Time to count 2 samples (A & B sides) - Counting 4 quadrants • 10–12 minutes	- Time to count 2 samples (A & B sides) • 2–3 minutes	- Time to count 2 samples (A & B sides) • ~1 minute

Figure 4. Comparison of hands-on steps between manual hemocytometer cell counting and cell counting using the Countess 3 Automated Cell Counter.

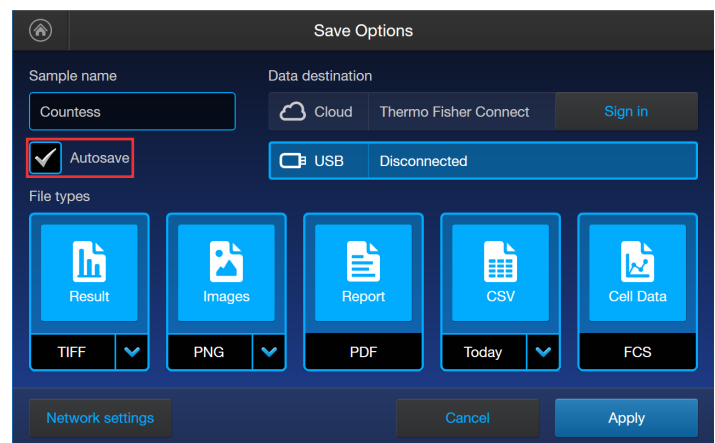
Rapid capture

Using the brightfield mode, once the slide is inserted, there is nothing further to do. The Countess 3 counter will automatically illuminate, focus, and count. This saves time by eliminating the need for additional menu selections to help get you quickly to results.



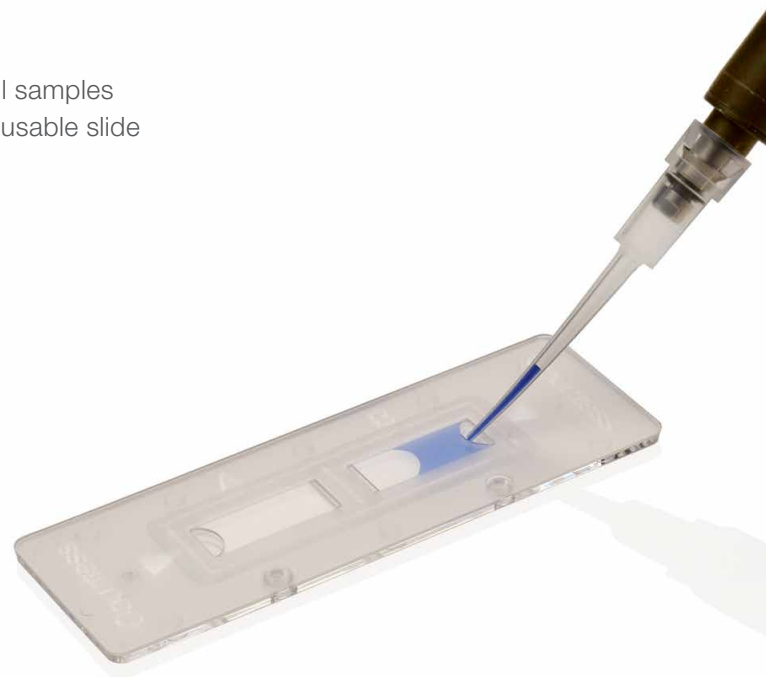
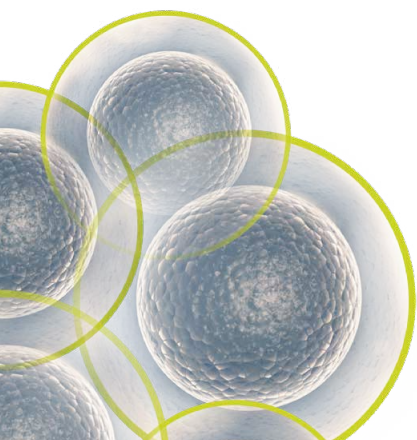
Autosave

By selecting the autosave feature, cell counts and data are automatically saved. Couple this with the rapid capture mode, and the cell counting and data retention process becomes fully automated.



Invitrogen™ Countess™ Cell Counting Chamber Slides

These disposable slides are convenient for quickly processing cell samples when there are many to process. There is no need to clean the reusable slide and wait for it to dry between cell samples.

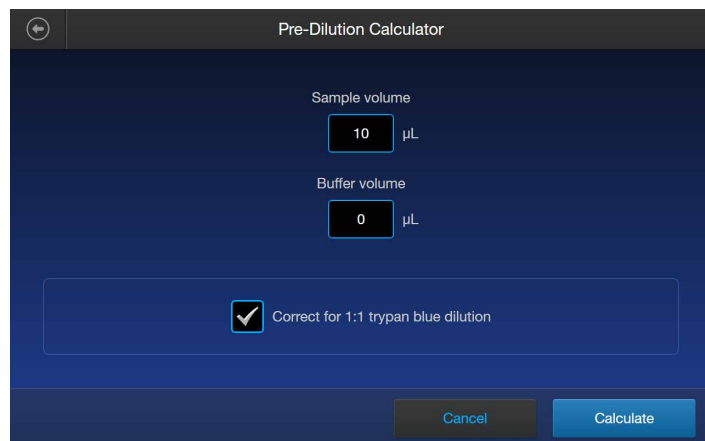


Convenience

On-board pre-dilution and cell splitting calculators make any necessary calculations seamless and integrated into the cell counting process. Viewing histograms and any additional desired gating of cell counts are easy to do on the Countess 3 cell counters, and a PDF report of your results can be saved and printed.

Pre-dilution calculator

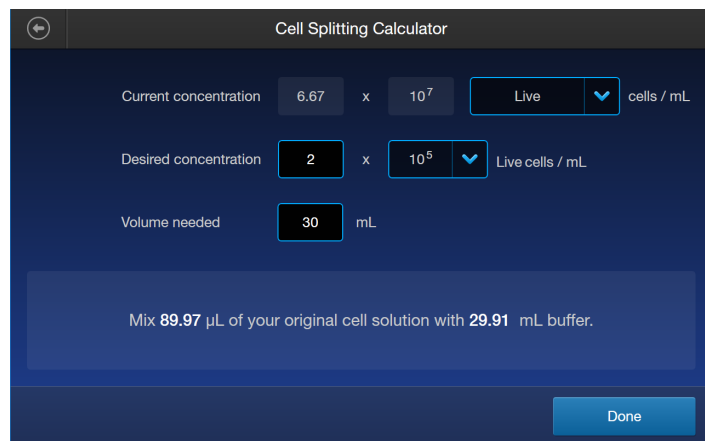
If the cell sample is concentrated and a dilution was required prior to cell counting, the Countess 3 cell counter can quickly and easily calculate the sample concentration. If Invitrogen™ Trypan blue solution was omitted from the count, the Countess 3 counter can adjust the dilution accordingly.



The Pre-Dilution Calculator interface shows input fields for Sample volume (10 µL) and Buffer volume (0 µL). A checkbox labeled "Correct for 1:1 trypan blue dilution" is checked. At the bottom are "Cancel" and "Calculate" buttons.

Cell splitting calculator

Easily determine the amount of cell sample and cell culture media/buffer needed for your experiment with the on-board cell splitting calculator. The cell count results are automatically used in the calculation; simply input the desired concentration and the volume needed.



The Cell Splitting Calculator interface shows input fields for Current concentration (6.67 x 10⁷ Live cells / mL), Desired concentration (2 x 10⁵ Live cells / mL), and Volume needed (30 mL). Below these fields, it displays the calculation: "Mix 89.97 µL of your original cell solution with 29.91 mL buffer." A "Done" button is at the bottom right.

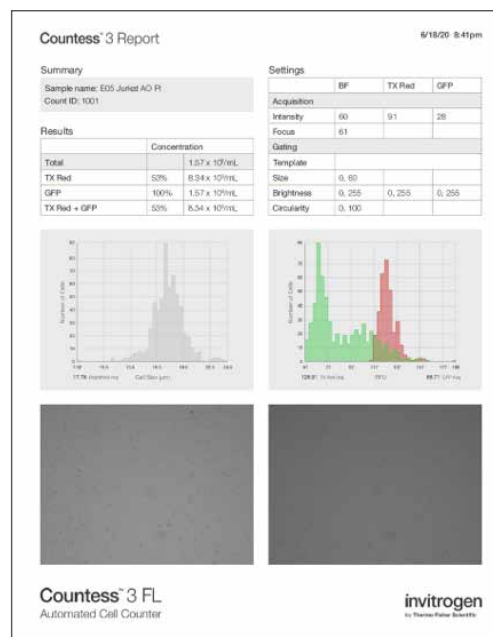
Easily gate cells and view histograms

Cells can be gated based on cell size, brightness, and circularity to fine-tune what is included or excluded for specific applications. View the histogram by selecting the graph icon, and watch the changes in real time as you use the gating features. The average cell size is also displayed on the histogram.



Printable report with cell counting results

Using a USB drive or Wi-Fi to establish cloud connectivity with the Countess 3 and 3 FL Automated Cell Counters, you can save your cell count results and images, and transfer them to your own computer. Images can be saved as TIFF, PNG, or JPG files, and the results are saved as a CSV file. A printable PDF report with results, images, and settings used can also be saved.



Responsive touchscreen and intuitive interface

Screen selections on the Countess 3 and Countess 3 FL Automated Cell Counters are intuitive and easy to navigate. The LCD screen is a responsive touchscreen and does not require an external computer. The 1,280 x 800 pixel screen provides high-quality cell images. The display is flush across the instrument making it easy to keep clean.

Countess 3 Reusable Slide

The Countess 3 Reusable Slide is an economical and sustainable choice. Both the Countess 3 and Countess 3 FL Automated Cell Counters can use the reusable slide with the reusable slide holder.



Wi-Fi-enabled cloud connection

The Countess 3 Automated Cell Counter can save files directly to the cloud-based Thermo Fisher™ Connect Platform. By connecting to a local Wi-Fi network with the Wi-Fi dongle, files can be saved to the cloud. Access these files at any time, inside the lab or out—for data that is available when and where you need it.



Countess 3 FL Automated Cell Counter

Fluorescence detection beyond GFP and RFP

The Countess 3 FL Automated Cell Counter offers one brightfield and two interchangeable fluorescence channels. The fluorescence colors detected are determined by the insertion of individual light cubes. The wide variety of light cubes available provides flexibility for multiple research applications from UV-excitatable DAPI to far-red.

Interchangeable LED light cubes

At the heart of the Countess system’s fluorescence technology are the unique Invitrogen™ EVOS™ Light Cubes. Each cube contains an LED, illuminating optics, and filters. Light cubes are user-interchangeable and automatically detected by the Countess 3 FL system for plug-and-play ease. There are ample light cubes to choose from.



Table 2. List of commonly used light cubes.

Light cube	Excitation (nm)	Emission (nm)	Common compatible dyes/fluorescent proteins
DAPI	357/44	447/60	DAPI, Hoechst, BFP
CFP	445/45	510/42	ECFP, Lucifer Yellow
GFP	470/22	525/50	GFP, Alexa Fluor 488, SYBR Green, FITC
YFP	500/24	542/27	EYFP, acridine orange (+DNA)
RFP	531/40	593/40	RFP, Alexa Fluor 546, Alexa Fluor 555, Cy3, DsRed, Rhodamine Red, dTomato
Texas Red	585/29	628/32	Texas Red, Alexa Fluor 568, Alexa Fluor 594, MitoTracker Red, mCherry
Cy5	628/40	692/40	Cy5, Alexa Fluor 647, Alexa Fluor 660, DRAQ5

See a complete list of available common and specialty light cubes at thermofisher.com/evoslightcubes

Table 3. Commonly used dyes and light cubes for use in the Countess 3 FL Automated Cell Counter for viability and apoptosis determination.

Dye	Light cube	Cat. No.
Viability dyes		
ReadyCount Blue Nuclear Stain	DAPI	A49904
ReadyCount Cell Viability Stain	GFP and Texas Red	A49905
ReadyCount Red Dead Cell Stain	RFP or Texas Red	A49903
ReadyProbes Cell Viability Imaging Kit, Blue/Green	DAPI and GFP	R37609
ReadyProbes Cell Viability Imaging Kit, Blue/Red	DAPI and RFP or Texas Red	R37610
LIVE/DEAD Viability/Cytotoxicity Kit	GFP and RFP or Texas Red	L3224
Propidium Iodide ReadyProbes Reagent	RFP or Texas Red	R37108
SYTOX Green Nucleic Acid Stain	GFP	S7020
SYTOX Red Dead Cell Stain	Cy5	S34859
7-Aminoactinomycin D (7-AAD)	Texas Red or Cy5	A1310
Apoptosis dye combinations		
ReadyCount Red Dead Cell Stain	RFP or Texas Red	A49903
CellEvent Caspases-3/7 Green Detection Reagent	GFP	C10423
SYTOX Red Dead Cell Stain	Cy5	S34859

Table 4. Cell lines verified on the Countess 3 Automated Cell Counters.

Cell	Animal	Organ
HeLa	Human	Cervix
U2OS	Human	Bone
A549	Human	Lung
CHO-K1	Hamster	Ovary
HEK293	Human	Kidney
SKBR-3	Human	Breast
BPAE	Bovine	Smooth muscle
HCASM	Human	Smooth muscle
Jurkat	Human	Blood
Ramos	Human	Blood
THP-1	Human	Macrophage
RAW	Mouse	Macrophage
MMM	Mouse	Macrophage
U937	Human	Blood
HepG2	Human	Liver
MCF-7	Human	Breast
Human PBMC	Human	Blood
Mouse PBMC	Mouse	Blood
Mouse hepatocyte	Mouse	Liver
CAR T	Human	Blood
Human hepatocyte	Human	Liver
Rat hepatocyte	Rat	Liver
Human splenocyte	Human	Spleen

Ordering information

Name	Quantity	Cat. No.
Countess 3 Automated Cell Counter		
Countess 3 Automated Cell Counter	1 instrument	AMQAX2000
Countess 3 Automated Cell Counter Starter Kit	1 kit	A49865
Countess 3 Automated Cell Counter w/ABRC Support	Instrument + 3 yr ABRC ext warranty	A49891
Countess 3 Automated Cell Counter w/Rapid Exchange	Instrument + 3 yr REX ext warranty	A49862
Countess 3 FL Automated Cell Counter		
Countess 3 FL Automated Cell Counter	1 instrument	AMQAF2000
Countess 3 FL Automated Cell Counter Starter Kit	1 kit	A49866
Countess 3 FL Automated Cell Counter w/ABRC Support	Instrument + 3 yr ABRC ext warranty	A49893
Countess 3 FL Automated Cell Counter w/Rapid Exchange	Instrument + 3 yr REX ext warranty	A49892
Accessories		
Countess 3 Cell Counting Chamber Slides	50 slides	C10228
Countess 3 Cell Counting Chamber Slides	500 slides	C10312
Countess 3 Reusable Slide	1 each	A25750
Countess 3 Reusable Slide Holder	1 each	A48208
Wi-Fi Dongle	1 each	A48209
ReadyCount Red Dead Cell Stain	100 tests	A49903
ReadyCount Blue Nuclear Stain	100 tests	A49904
ReadyCount Cell Viability Stain	100 tests	A49905

Find out more at thermofisher.com/countess

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