

# Countess II Automated Cell Counters

## Frequently asked questions

### General use

**Q. What size of cells are the Invitrogen™ Countess™ II Automated Cell Counter and Invitrogen™ Countess™ II FL Automated Cell Counter able to count?**

A. Objects ~4–60 µm can be counted. Cells ~7–60 µm can be counted and assessed for viability with brightfield viewing.

**Q. How long do the instruments take to count?**

A. ~10 seconds per sample.

**Q. What is the counting range?**

A. The Countess II instruments are designed to read samples with concentrations in the range of  $1 \times 10^4$  cells/mL to  $1 \times 10^7$  cells/mL.

**Q. Why do my brightfield and fluorescent counts differ by 2x?**

A. When counting in brightfield mode, the Countess II instruments automatically assume a 1:1 dilution has been made with trypan blue. The same assumption is not made when in fluorescence mode.

**Q. What count-to-count precision should be expected?**

A. When an experienced worker manually counts cells using a glass hemocytometer together with a microscope, count-to-count variability of a single sample is commonly 10% or more. Users of Countess II Automated Cell Counters should typically see <5% count-to-count variability.

**Q. Can I use the light cubes from my Invitrogen™ EVOS™ imaging system in the Countess II FL instrument?**

A. Yes, the Countess II FL instrument uses the same light cubes as the EVOS imaging systems.

**Q. Can I buy the Countess II FL instrument without light cubes?**

A. Yes, the Countess II FL instrument without light cubes installed will function exactly like the Countess II instrument—as a brightfield cell counter. Customers can easily add light cubes as application need evolves.

**Q. Can I only use the reusable slide on the Countess II FL instrument, or can I use it on the Countess II instrument as well?**

A. The reusable slide is only compatible with the Countess II FL instrument.

**Q. Can I show both live/dead and fluorescent results using the same count and a single display?**

A. No, in order to obtain brightfield viability and fluorescence information, two separate counts have to be performed.

**Q. What is the fluorescence sensitivity limit for the Countess II FL instrument?**

A. As fluorescence is measured on a relative scale, this is too environmentally dependent to offer a quantitative answer. In short, the Countess II FL instrument offers fluorescence sensitivity similar to that of a low-magnification epifluorescence microscope.

## Cell counting capabilities

**Q. What cell types have been tested?**

A. HEK 293, A431, CHO-M1, CHSE, COLO-205, COS-7, HeLa, HepG2, HL-60, J774(MMM), Jurkat, K-562, MCF-7, MRC-5, NIH/3T3, PC-12, SF-21, U266, and U2OS. Primary cells: human adipose tissue-derived stem cells, HASMCs, HPAECs, HPASMCs, HUVECs, C2C12, RBCs, and PMBCs. We are continually testing additional cell types.

**Q. Can the Countess II FL instrument determine transfection efficiency?**

A. Yes, the instrument can count in both fluorescence and brightfield modes, allowing simple calculation of transfection efficiency that is shown on the results screen as “% positive” for the light cube of interest.

**Q. Can the Countess II instruments count bacterial cells?**

A. No, bacteria are too small to be distinguished from non-cell debris.

**Q. Can the Countess II instruments count irregular or elongated cells?**

A. Yes, but you may need to experiment with several circularity settings until you find the one that is best for your cell type.

**Q. Are the Countess II instruments able to accurately count clumps of cells?**

A. The advanced counting algorithms of the Countess II instruments can clearly identify cell boundaries within clumps of cells, resulting in accurate cell counts.

**Q. Can the Countess II instruments count peripheral blood mononuclear cells (PBMCs)?**

A. Yes, the Countess II instruments can count PBMCs. However, it cannot differentiate white blood cell types.

**Q. Can the Countess II instruments count red blood cells (RBCs)?**

A. Yes, the Countess II instruments can count RBCs. However, we recommend diluting the blood sample approximately 1:10,000. The instrument cannot assess the viability of RBCs due to their pigmentation and trypan blue staining pattern.

**Q. Can the Countess II instruments count sperm cells or other fast-moving cells?**

A. The instrument does not count live sperm cells or other fast-moving cells such as protozoa.

**Q. How will the Countess II instruments discriminate a dividing cell?**

A. If there are two cells attached to each other with enough circular definition for each, the image analysis firmware will distinguish them as two different objects.

**Q. How do the Countess II instruments distinguish live and dead cells?**

A. Dye exclusion using 0.2% trypan blue dye. Trypan blue is excluded by the live cells and enters the dead cells.

**Q. Do I have to set specific parameters for specific cell types?**

A. We have tested over 20 commonly used cell types, including primary cells, PBMCs, insect cells, and fish cells, using the default settings. Specific cell types could require some parameter setting adjustments, and those may be optimized by the user.

**Q. Why do most of my cells look dead?**

A. There are two predominant reasons for this: either the cells in question are truly dead or the focus should be adjusted. In some cases, heavily pigmented cells will also look dead in brightfield; such cells may require fluorescent viability stains.

**Q. What cell types work best on the Countess II instruments?**

A. The Countess II instruments were optimized for mammalian cell counting; however, counting other cell types may be possible.

## Dyes for the Countess II instruments

**Q. Can other dyes besides trypan blue be used with the Countess II instruments?**

A. So far, we have not found any alternative dyes for brightfield viability measurements that can be used with the Countess II instruments. However, many fluorescent stains or proteins can be visualized on the Countess II FL instrument, provided the correct light cube is installed.

**Q. Are there specific dyes that work well for measuring viability on the Countess II FL instrument?**

A. The Countess II FL instrument is able to work with dyes that you may already be using to measure viability. Some popular dyes that have been validated on the Countess II FL instrument are included in the table below.

Viability dye	Light cube	Cat. No.
ReadyProbes™ Cell Viability Imaging Kit, Blue/Green	DAPI; GFP	R37609
ReadyProbes™ Cell Viability Imaging Kit, Blue/Red	DAPI; RFP or Texas Red™	R37610
LIVE/DEAD™ Viability/Cytotoxicity Kit	GFP; RFP or Texas Red	L3224
Propidium Iodide ReadyProbes™ Reagent	RFP	R37108
SYTOX™ Green Nucleic Acid Stain	GFP	S7020
SYTOX™ Red Dead Cell Stain	Cy®5	S34859
7-Aminoactinomycin D (7-AAD)	Texas Red or Cy5	A1310

**Q. Are there specific dyes that work well for measuring apoptosis on the Countess II FL instrument?**

A. The Countess II FL instrument is able to work with dyes that you may already be using to measure apoptosis. Some popular dyes that have been validated on the Countess II FL instrument are included in the table below.

Apoptosis dye	Light cube	Cat. No.
CellEvent™ Caspase-3/7 Green Detection Reagent	GFP	C10423
SYTOX Red Dead Cell Stain	Cy5	S34859

## Physical features

**Q. What are the weight and dimensions of the Countess II instruments?**

A. Both the Countess II and Countess II FL instrument weigh 3.6 kg (8 lb). Dimensions (W x D x H) of both instruments are 23 cm (9 in) x 14 cm (5.5 in) x 23 cm (9 in).

**Q. How do I clean and maintain the instrument?**

A. Clean the surface of the instrument with a damp cloth. Wipe the screen dry immediately. The Countess II instruments have no moving parts to maintain, no tubes to clean, and no buffers to replace.

**Q. Is a computer required?**

A. No.

**Q. Can a computer be hooked up to the cell counter?**

A. No, but you can use a USB drive to easily transfer your data and images to a computer.

**Q. Do I need to load software on my computer?**

A. Special analysis software is not needed for opening data or image files.

**Q. Can the images be saved?**

A. Yes, the result and image files can be saved after each count. Images can be saved as JPG, PNG, TIFF, and BMP files.

**Q. Why can't I save my images and data?**

A. Ensure a FAT-formatted USB drive is inserted in the instrument.

**Q. How often do I have to calibrate the Countess II instruments?**

A. The Countess II instruments come precalibrated. You do not have to calibrate the instruments.

Find out more at [thermofisher.com/countess](http://thermofisher.com/countess)

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