

The Countess™ Automated Cell Counter FAQs

Basic technical questions

- Q How long does it take to count?
- A 30 seconds per sample
- Q What is the counting range?
- A The Countess™ Automated Cell Counter is designed to read samples from 1×10^4 cells/mL to 1×10^7 cells/mL, with the highest accuracy between 1×10^5 cells/mL and 4×10^6 cells/mL
- Q What size cells does it count?
- A 5 μ m cells to 60 μ m cells
- Q What does it use to distinguish live and dead cells?
- A Dye exclusion using 0.2% trypan blue dye. Live cells exclude the dye. Dead cells take it up into their cytoplasm.
- Q How does it count the cells?
- A A sophisticated image analysis algorithm identifies objects, classifies cells by roundness and size, and then distinguishes live cells from dead cells by their staining pattern.
- Q Does it measure cell size?
- A Yes. It measures the size of live and dead cells as well as the average size of each cell population.

Physical features

- Q What are the dimensions and weight of the counter?
- A The Countess™ Automated Cell Counter has a very small footprint (27 cm (w) x 20 cm (d) x 19 cm (h)) and weighs about 5.7 kg.
- Q Can the slides be packaged in a nonsterile format and not individually wrapped?
- A Currently, we only supply the slides individually wrapped.
- Q How do I clean and maintain the instrument?
- A Clean the surface of the Countess™ instrument with a damp cloth. To clean the LCD screen, turn off the Countess™ instrument, disconnect the power cable, and clean the LCD screen with a soft cloth lightly moistened with LCD cleansing detergent. Cleaning the screen with excessive force can damage the LCD screen. Wipe the screen dry immediately. Do not reuse the counting chambers.
- The Countess™ Automated Cell Counter does not need regular maintenance. To troubleshoot problems with Countess™ cell counter, contact Technical Support (page 24 of the manual). To avoid damaging the instrument, do not perform any repairs or service on the Countess™ instrument.

Computer compatibility and archiving

- Q Does it require a computer?
- A No
- Q Can you hook the counter up to a computer?
- A No. You can use a USB drive to transfer your data from the counter to a computer that uses a Windows operating system.

Computer compatibility and archiving, continued

- Q Do I need to load software on my computer?
- A For maximum analysis capabilities and to print a report, you can load Countess™ PC software on your computer. The Countess™ PC software is available free from www.invitrogen.com/countess. It is also provided on the USB drive that comes free with the instrument. This software allows you to manipulate your cell-counting data and to save your data in a pdf report format for printing or archiving.
- Q Does the counter save images?
- A Yes. All of the data is saved as a .DBT file that can be opened with the Countess™ PC software. This also includes a .JPG file of the image. You can open the .JPG file with any imaging software.
- Q Can the counter generate a report?
- A Yes. You will need to use the Countess™ software on your PC to generate the report. The report shows an image of your cells, all numerical data, and a cell size histogram.
- Q Do I need special software to see my results saved to a thumb drive on my computer?
- A You can open the .CSV file with any spreadsheet software. You can also open the image file with any imaging software. To see the complete data set, manipulate the data and print a report, you may use the Countess™ PC Software available free at the Invitrogen Countess™ website.

Cell counting capabilities

- Q What cell types have you 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, HASMC, HPAEC, HPASMC, HUVEC, C2C12, RBC, and PMBC. We are continually testing additional cell types. See the complete list and data on the Invitrogen Countess™ website at www.invitrogen.com/countess.
- Q Can the Countess™ instrument count irregular or elongated cells?
- A Possibly. We have not tested very irregular or elongated cells. If the cells are very irregular or elongated, try the “Parameters” function under the “Settings” menu to vary the circularity. This function alters the way that the image analysis software recognizes cells. You may need to experiment with several circularity settings until you find the one that is perfect for your cell type.
- Q Can the Countess™ instrument count yeast cells?
- A We have successfully counted *Saccharomyces cerevisiae* (Fleishman’s baker’s yeast), a consumer product. The Countess™ instrument successfully counted these yeast cells, as long as they were not too clumpy. However, it cannot distinguish viability.
- Q Can the Countess™ instrument count bacterial cells?
- A No. The bacteria are too small to be distinguished from non-cell debris.
- Q How does the Countess™ instrument deal with clumps of cells?
- A The Countess™ instrument can accurately count clumps of up to about five or six cells. Above this number, cells will overlap and the Countess™ instrument seems to be about as accurate as the human eye. However, the cells will be counted as larger than real size.

Cell counting capabilities, continued

Q Can the Countess™ instrument count white blood cells?

A The Countess™ instrument was able to count white blood cells from lysed whole blood and Ficoll cell preparations. You can download the application note [here](#).

Q Can the Countess™ instrument count whole blood cells containing non-lysed cells?

A Yes. You should dilute the blood sample by approximately 1:10,000 and count in “bead” mode. The instrument cannot assess the viability of cells in a whole blood sample.

Q Can the Countess™ instrument count PBMCs?

A Yes. The Countess™ instrument can count PBMCs. However, it CANNOT differentiate white blood cell types.

Q Will the Countess™ instrument count RBCs (red blood cells)?

A Yes. The Countess™ instrument can count RBCs. Dilute the blood sample by approximately 1:10,000 and count in “bead” mode. The instrument cannot assess the viability of red blood cells.

Q Can the Countess™ instrument count plant cells?

A We have not tested plant cells. If the plant cells fall within the optimal size range for the instrument and are not extremely clumpy, the Countess™ instrument should be able to count plant cells.

Q Can the Countess™ instrument count sperm and other fast moving cells?

A It is difficult to count cells that are moving quickly. The instrument does not count live sperm and fast-moving protozoa.

Q Can the Countess™ instrument count hepatocytes?

A The instrument is able to calculate total concentration, but CANNOT provide viability information on the cells. Set the instrument to “bead” mode, then you can perform the cell counting.

Q Can parameters be set such that if one has a set of mixed cells and only the lymphocytes need to be counted, only those cells are counted by the Countess™ instrument?

A No. The instrument cannot selectively analyze a specific size population. The instrument takes an average of all the objects in the image and determines the average cell size. Then it sets limits above and below that average size based upon the adjustable settings.

Q How will the Countess™ instrument discriminate a dividing cell?

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

Q Do I have to set my 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 have some parameter setting adjustments, and those may be optimized by the user.

Dyes and calibration

Q What dyes can be used with the Countess™ instrument?

A We have only tested the Countess™ instrument with trypan blue.

Q Can other dyes besides trypan blue be used with the Countess instrument?

A So far, we have not found any alternative dyes that can be used with the Countess™ instrument. We have tested eosin Y, eosin B, and nigrisin. However, we have determined that a lower concentration of trypan blue (0.1%) does work with the instrument, which can help to minimize contact with trypan blue. You can download the application note [here](#). Please [contact us](#) if you have a suggestion for another dye that we should test.

Q How often do you have to calibrate the Countess™ instrument?

A The Countess™ instrument comes precalibrated. You have to calibrate if you update the firmware on the instrument. You also have to recalibrate the instrument if you decide to use a different brand or concentration of trypan blue than is supplied with the kit. Calibration simply sets the background to the correct blue color and takes approximately 2–3 minutes. Other than these two situations, you do not have to calibrate the instrument.

Q What concentrations of trypan blue will work?

A We have tried trypan blue concentrations from 0.05–0.6%, and they all still work, as long as the instrument is recalibrated for that concentration of trypan blue.

Q Can I set my dilution factors in the setting?

A No. The sample preparation was simplified to 1:1 mix, load, read, and the result given only takes that 1:2 dilution into account. Any further dilutions for a final answer need to be calculated by the user.