

Red One™: Frequently Asked Questions (FAQ)

The following FAQs are providing first answers to the users of Red One™.

For more detailed insights, please refer to the user guide provided with Red One™.

Red One™ Basics

Q. What kinds of cells can I detect and count with Red One™?

A. Red One™ detects **viable microorganisms** including bacteria, yeasts and molds.

Q. What kind of samples can I analyze with Red One™?

A. **Filterable samples** can be analyzed. This includes waters, buffers, liquid culture media, juices, milk...

Q. Does it require any specific sample preparation?

A. No specific sample preparation is required before using Red One™. Traditional sample preparation methods remain valid to get the sample filterable (dilution in buffer or liquid medium for example).

Q. What is Red One™ time-to-result?

A. Single cells detection result (counting) is delivered **within 10 minutes** once the sample has been dropped on the cap.

Q. What is the minimum size Red One™ can detect?

A. Cells **over 0.3 µm** can be counted.

Q. What is the Red One™ counting range?

A. Red One™ can count samples with concentrations in the range of **10 to 5x10⁵ microorganisms** per cap (linear range).

Q. What volume can be filtered on one cap with Red One™?

A. **Nominal volume is 1 mL**. Red One™ can filter volumes from 10 µL to 100 mL according to specific protocols.

Q. How does Red One™ count cells?

A. Red One™ counts **fluorescence-stained microbial cells**. The evolution of fluorescence over time ("**staining kinetics**") is analyzed automatically by an algorithm, allowing to differentiate viable cells from inert particles.

Q. Can I use Red One™ under a laminar flow hood?

A. Yes you can.

Dimensions are **25*45*60cm** (with drawer opened). It easily fits into a laminar flow hood. Two cords (power and communication) have to get outside and connect to the computer for analysis.

Q. How often do I have to calibrate Red One™?

A. Red One™ does not require calibration. Redberry recommends a maintenance check once a year.

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Consumables – Red Caps

Q. Is Red Cap single-use or reusable?

A. Each cap is **single-use only**. A sterile seal has to be removed before use.

Q. Is Red One™ compliant with other consumables?

A. No.

Q. How do I manage caps after use?

A. Red Caps have to be treated after use **as other standard biological waste** (following standard rules for infectious waste management).

Q. What are the characteristics of the filtration membrane?

A. Polyester (PET) membrane.
Membrane diameter: 21 mm.
Porosity: 0.3 µm.

Running analysis with Red One™

Q. What should I do before switching on Red One™?

A. You must ensure that the two cords are properly plugged at the back of the equipment. Check that power supply is properly plugged on a standard 220V power plug. Check that the connection wire is properly connected to the computer (USB). Agitate the bottle of dye, place it on the left drawer of the equipment and place the plug on its neck, ensuring the metal pipe is properly dipped in the liquid. Check that the waste filtration bottle is properly connected to the equipment (back side – brass connector).

Q. How do I switch on Red One™?

A. On/OFF button at the back. Once switched on, red lights appear on the front of the machine (low part).

Q. How can I launch an analysis?

A. Fix your cap on the baseplate. Click on the play button located on the top left corner of the screen, complete your ID-sample and enter the volume to be analysed. Then, drop your sample onto the cap and click on “start analysis”. Close the drawer.

Q. Can I stop an analysis?

A. Yes you can, by clicking on the stop button, next to the play button on the top left corner.

Q. What should I do after an analysis?

A. You have to open the drawer on the right-hand side and remove the cap. System is then ready for a new analysis.

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Dyes/Detection

Q. What is the action mode of Red One™ TVC (Total Viable Count) Staining Dye?

A. Red One™ TVC Staining Dye is an **esterase-sensitive fluorophore**. Cellular esterases present in a viable cell hydrolyze the dye turning it into a fluorescent compound which is retained in the cell.

These enzymes are present in cells showing an active metabolism. For instance, viable yeasts, bacteria, molds and eukaryotic cells can be labelled.

Q. What is its level of toxicity?

A. It is **not toxic**. As for any microbiological test, you should wear standard protections (lab coat, gloves).

Q. What if I want to use another dye?

A. Dyes offered with Red One™ are guaranteed to deliver the best results for your applications. They are compatible with Red One™ wavelengths - Excitation: 492 nm (blue), Emission: 520 nm (green). Specific wavelengths available on request.
Other dyes could be used upon request to Redberry.

Q. How long can I store and use the dye?

A. The dye is delivered in a preparation kit containing all required components.
This kit can be stored for **3 months at 5°C before reconstitution**.
Once prepared, the dye can be used **within 12 hours**.

Q. Can I use LIVE/DEAD BacLight Bacterial Viability Kits?

A. No. The dyes used in these kits are not compliant with Red One™ (specific wavelengths required).

Optical sensor

Q. What kind of image sensor is used in Red One™?

A. Red One™ is based on a High Resolution camera (CMOS sensor resolution >100 Mpx).

Q. How many fields of view are captured for image analysis?

A. The whole surface of the membrane is captured for each picture. Red One™ does not rely on any scanning device nor image reconstruction software.

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Cell counting

Q. On which set of microorganisms has Red One™ been validated?

A. Reference Pharmacopea germs: *Escherichia coli* (ATCC8739), *Staphylococcus aureus* (ATCC6538), *Pseudomonas aeruginosa* (ATCC9027), *Methylobacterium extorquens* (ATCC15911), *Bacillus subtilis* (ATCC6633), *Candida albicans* (ATCC10231), *Aspergillus brasiliensis* (ATCC16404).

The Red One™ counter has also been successfully tested for Total Viable Count on agri-food matrices (lettuce, steak, cheese, bread yeast) and drinking water.

Q. Can Red One™ count both gram negative and positive cells?

A. Yes, Red One™ can count both gram negative and positive cells.

Q. Can Red One™ count viable cells?

A. Yes. Viable cells can be differentiated from inert particles, based on the analysis of the **fluorescence evolution over time** (“staining kinetics”) emitted by an esterase-sensitive fluorophore.

Q. Is there any available option to count only cultivable cells with Red One™?

A. Yes, for potable waters mainly. With specific parameters, Red One™ can focus its detection on cultivable cells, meaning cells with remarkable metabolism.

Q. Can Red One™ be used for mycoplasma testing?

A. No. Mycoplasma are too small (down to 0.1 µm) to be retained on the Red Caps PET membrane.

Q. Can Red One™ detect viruses?

A. No. Viruses are too small (from 10 to 300 nm) to be detected with Red One™.

Q. Can Red One™ count irregular or elongated cells?

A. Yes it can. Counting is based on staining kinetics. No morphologic differentiation is performed.

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Users and Data

Q. How do I get results from Red One™?

A. **Images and data are automatically saved.** Results (counts) are displayed on the HMI (Human Machine Interface – on the computer). You can download an **analysis report on request.**

Q. How are the results displayed on the HMI?

A. Viable cells and inert particles are identified and counted on the HMI (top left corner).

Q. Is a computer required?

A. Yes. Red One™ is delivered with the computer, a screen, mouse, keyboard and a set of wires to connect the whole platform.

Q. I want to see past analysis results and I cannot find them. Where are they?

A. Your results are automatically saved by the system. To see a past analysis, chose the sample ID on the menu through the HMI (Human Machine Interface – on the computer). Analysis reports are saved in the folder selected with the HMI – see icon on top of it.

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

A. The platform is **ready to use.** You do not need to download any software by yourself.

Q. Is the platform compliant for multi-users?

A. Yes. Each user can access the software by connecting to his/her personal account (with login and password). Accounts can be created by authorized personnel.

Q. Does Red One™ require an Internet connection?

A. **No.** All data and analysis are stored and performed locally. Remote access is possible as an option.

Q. Is Red One™ software compliant with 21 CFR Part11?

A. 21 CFR Part 11 version of Red One™ software is available for pharmaceuticals users.