Technology that counts

chemometec

Quick Guide

Viability and Cell Count, Vitality and Cell Cycle Assay in one instrument!

NucleoCounter® NC-250™

How to get started

Dear NucleoCounter® Customer,

Thank you for purchasing the NucleoCounter® NC-250™ which offers not only fast and precise Viability and Cell Count Assays, but also fast and easy assaying of cell cycle profiles with top class precision and the fastest apoptosis assay!

The NucleoCounter® NC-250™ Concept

- ✓ 8 viability and cell counts in less than 3 minutes
  - Total cell count
  - Viability
  - Cell diameter
  - % of cells in clumps
- ✓ Up to 8 advanced analyses in one run
  - Fast and easy - High precision cell cycle analysis
  - Cell health determination via the fastest apoptosis assay

- ✓ Fast and easy operation
- ✓ Excellent reproducibility
- ✓ No calibration
- ✓ Maintenance and service free
- ✓ 21 CFR Part 11 ready

NucleoView™ Software
Included on a USB stick for an unlimited number of installations.

NC-Slides A8™ and A2™
A8-Slides™ for fast analysis and A2-Slides™ for high precision.

Solution 18
For Viability and Cell Count Assays.

How to get started - 8 easy steps to install the NC-250™

1. Unpack the NC-250™ instrument and plug it in the main outlet. **Do NOT connect the USB cable to the PC.**
2. Make sure that there are full administrator rights on the PC during the installation of the NucleoView™ NC-250™ software.
3. Insert the USB stick in the PC and open the “Install_Guide.html” file for detailed installation instructions.
4. Open the “Install NucleoView NC-250 X.X.X.X.exe” file (the Xs indicate the version number e.g. 1.0.22.0). **Do NOT open the .bin file.**
5. Follow the instructions on the screen. After the software installation it will be required to restart the PC.
6. After the restart the NucleoView™ NC-250™ software will automatically open and finish the installation.
7. Follow the on-screen instructions to install the instrument.
8. The NucleoCounter® NC-250™ is ready to use when the LED indicator light on the instrument turns green.

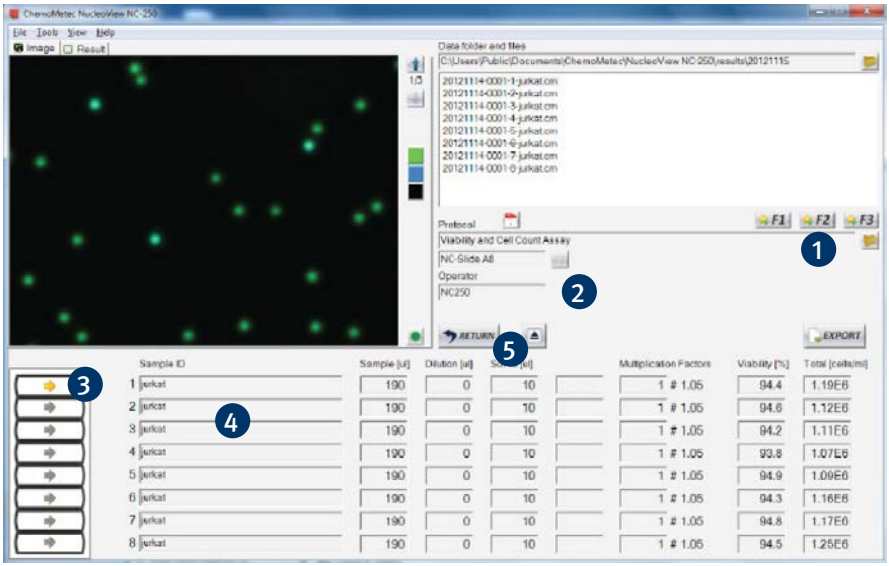
For detailed instructions, please read the manual.

How to perform Viability and Cell Count Analysis

Fast and Precise Cell Count and Viability Assays!

The NucleoCounter® NC-250™ from ChemoMetec is a step forward for automated cell counting.

1. Select the desired protocol from the menu: for example, '**Viability and Cell Count Assay**'
2. Choose the media type (NC-Slides A8™ or A2™)
3. Select the chamber(s) to analyse
4. Optional: Enter Sample ID and Operator Name
5. Mix the cell sample with the assay defined solution(s), load the NC-slide and press 'RUN'



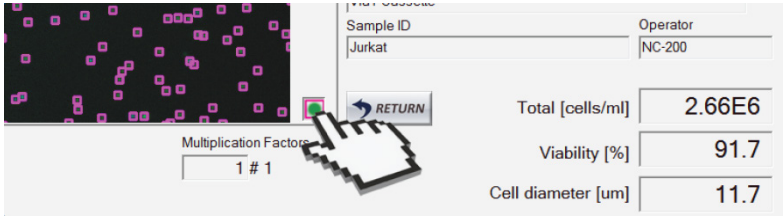
TIP: Find the application note for the selected protocol by clicking the icon.

Optional: Visual Inspection of Counting Gates

OPTION 1 (PREFERRED)

Click the green dot in the right-hand corner of the image window.

This activates the image overlay function indicating all the events in the total cell count. The mouse scroll button allows the user to zoom in at the cursor position. Cells will be framed by a pink square.

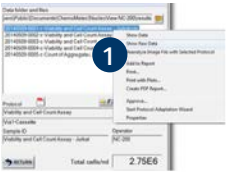


OPTION 2 (ADVANCED)

1. Right-click on the sample file name in the 'Data folders and files' window and select 'Show Raw Data'.

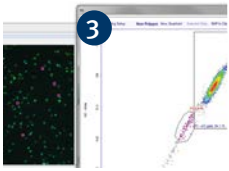
   A new window will open displaying scatter plots and histograms of event intensity and area for the appropriate channels (Acridine Orange and DAPI).

   It is important that the centre of the population most usually seen as a colored region on the scatter plot, is included in the counting gate.



2. To check distinct cell populations, create a new polygon around the particular cell population.

3. Right-click inside of the newly formed polygon and select 'Add Cells Inside Gate to Image Overlay'. This activates the image overlay function indicating all the events visually to determine the validity of their inclusion or exclusion from the final counting result.



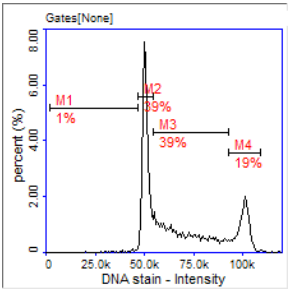
4. Delete the polygon and the image overlay by right-click and selecting 'Delete Image Overlay'.

How to Perform a 2-Step Cell Cycle Assay

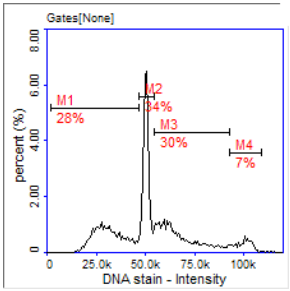
The NucleoCounter® NC-250™ provides fast and easy determination of cell cycle profiles with top class precision.

1. Add 20 µl **Solution 12** to 980 µl **Solution 10** and mix
2. Make a cell pellet of $5x10^5$ to $1x10^6$ cells
3. Add 250 µl of the **Solution 10** and **Solution 12** mixture to your adherent cells or cells in suspension and mix
4. Incubate at 37°C for 5 minutes
5. Add 250 µl of **Solution 11** and mix
6. Select the protocol from the menu: '**2-Step Cell Cycle Assay**' and choose the media type (NC-Slide A8™ or NC-Slide A2™)
7. Select the chambers to perform the assay on
8. Optional: Enter Sample ID and Operator Name
9. Load the NC-Slide A8™ or NC-Slide A2™ and Press 'RUN'
10. Add markers to the DNA content histogram to distinguish between the cells in the different cells cycle stages.

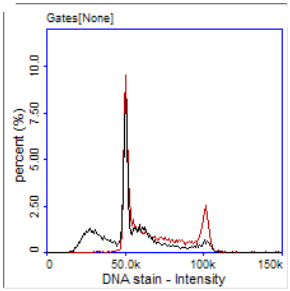
Untreated HeLa cells



Camptothecin treated HeLa cells



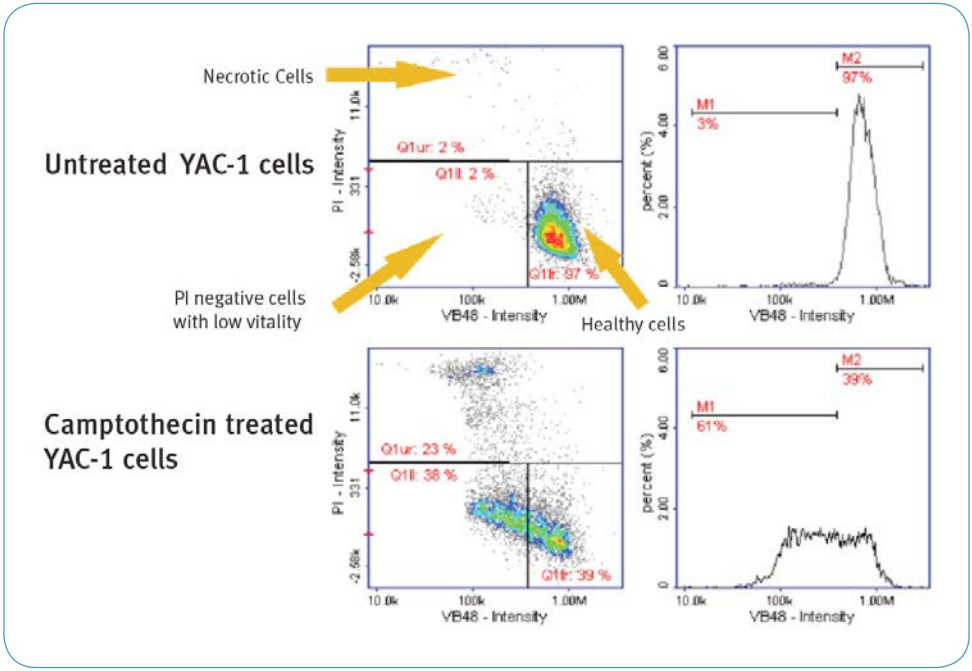
Merged



How to Perform a Vitality Assay

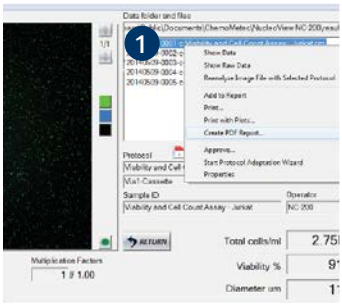
The NucleoCounter® NC-250™ provides the world's fastest Vitality assay. **Vitabright48™** is a component of **Solution 6** instantly fluoresces when it binds thiols inside cells. The level of thiols is a direct measurement of cell vitality.

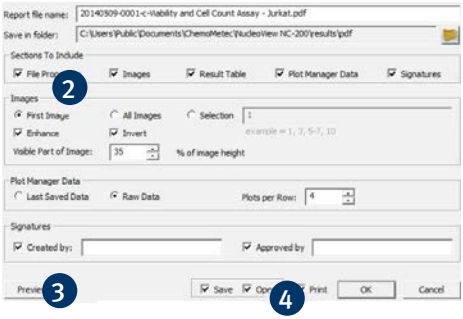
1. Select the protocol from the menu: '**Vitality Assay**'
2. Choose the media type (NC-Slide A8™ or NC-Slide A2™)
3. Select the chambers to perform the assay on
4. Optional: Enter Sample ID and Operator Name
5. Mix the cell sample with **Solution 6** (in a ratio 19:1), immediately load the NC-Slide A8™ or NC-Slide A2™ and press 'RUN'
6. If required, the percentage of the different cell populations can be investigated by adding a quadrant or markers to the scatter plot or histogram, respectively



PDF Reports

1. Right-click on file to create a PDF report





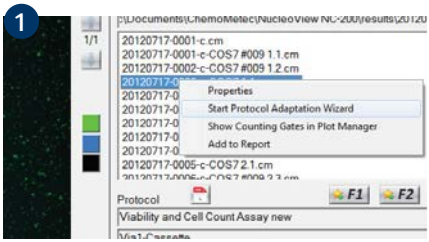
2. Select the parameters to be visible on the report, and the properties of the parameters

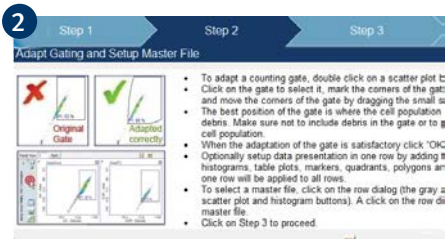
3. Optional: Preview your result

4. Save and/or print your report to the default printer

Tip: Batch exports can be done from the NucleoView™ File Browser.

Only if Required: Create your own version of a protecol with Adjusted Generic Gates





1. Perform the desired type of assay on a sample of the cells to be analyzed. In the Tools menu select Protocol Adaptation Wizard or right-click on the desired file and select 'Start Protocol Adaption Wizard'.

2. Follow the instructions in the Protocol Adaptation Wizard to create your own adapted protocol.

Note: The viability and cell count results will not be adjusted for the image file used for the adaption of counting gates. New results can only be obtained by running a new sample with the new gating protocol.

Additional Resources

Go to **www.chemometec.com** to find:

- Documentation
- Safety Data Sheet
- Application notes
- Certificates of analysis
- Videos etc.

Consumables/kits:

Item no.	Description
942-0003	NC-Slides A8™
942-0001	NC-Slides A2™
910-3003	Solution 3
910-3006	Solution 6
910-3010	Solution 10
910-3011	Solution 11
910-3012	Solution 12
910-3017	Solution 17
910-3018	Solution 18
910-0003	Reagent A100
910-0002	Reagent B
912-0012	NucleoCounter® NC-250™ IQ/OQ Kit
974-0004	PQ Package for NucleoCounter® NC-250™

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chemometec

ChemoMetec A/S
Gydevang 43, DK-3450 Allerød - Denmark

Phone	(+45) 60 23 46 61
Fax	(+45) 48 13 10 21
Mail	contact@chemometec.com
Web	www.chemometec.com