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# TC20™ Automated Cell Counter

## Instruction Manual

Catalog #145-0101





# **TC20™ Automated Cell Counter**

**Instruction Manual**



## Bio-Rad Laboratories Resources

Table 1 lists Bio-Rad resources and how to locate what you need.

**Table 1. Bio-Rad resources.**

Resource	How to Contact
Local Bio-Rad Laboratories representatives	Find information and contacts on the Bio-Rad Laboratories website by selecting your country on the Home page ( <a href="http://www.bio-rad.com">www.bio-rad.com</a> ). Find the nearest international office listed on the back of this manual
Technical notes and literature	Go to the Bio-Rad Laboratories website ( <a href="http://www.bio-rad.com">www.bio-rad.com</a> ). Type a term in the Search box and select <b>Documents</b> to find links to literature
Technical specialists	Bio-Rad Laboratories Technical Support scientists provide our customers with practical and expert solutions. To find local Technical Support on the phone, contact your nearest Bio-Rad Laboratories office. For Technical Support in the United States and Canada, call 1-800-424-6723 (toll-free phone), and select the Technical Support option

## Warranty

The TC20™ automated cell counter and associated accessories are covered by a standard Bio-Rad warranty. Contact your local Bio-Rad Laboratories office for the details of the warranty.

## Safety Use Specifications and Compliance

A **Warning!** label in this manual warns you about sources of injury or harm, including risk of electrical shock.

**Warning!** Do not attempt to repair or remove the outer case of the TC20 automated cell counter or other accessories. If you open this instrument, you put yourself at risk for harm to body or equipment from electrical shock.

This instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards.

**Table 2. Environmental conditions for safe operation of the TC20 automated cell counter.**

Transient category	Installation categories II
Operating power	90–240 VAC, 1.2 A
Frequency	50/60 Hz
Electrical input	12 VDC, 2.5 A
Installation site	Indoor use only
Operating temperature	10–35°C
Maximum relative humidity	20–80%
Altitude	<2,400 m
Pollution degree	2
Degree of protection	IPX0

**Note:** Do not store or operate the unit near a sink as contact with water could cause electric shock.

Unit is heavy, do not store or operate it at the edge of a laboratory bench. During operation unit must be in upright position.

Do not tip the unit over if the counting slide is inserted in the slide port; this could cause accidental spillage of sample and contaminate the unit.

Use only Bio-Rad counting slides, using other manufacturers' slides could damage the unit.

TC20 cell counter is factory calibrated, no further calibration is needed.

## Safety Compliance

This instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- EN61010-1 — Electrical Equipment for Measurement, Control, and Laboratory Use
- UL Std No. 61010A-1 — Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- UL Std No. 61010A-2-010 — Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- CAN/CSA C22.2 No. 1010.1-92 — Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements (includes Amendment 1)
- CAN/CSA C22.2 No. 1010.1B-97 Amendment 2 CAN/CSA C22.2 No. 1010.1-92 — Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- IEC 61010-1 — Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1 General Requirements

## Electromagnetic Compatibility (EMC)

- F.C.C. Title 47 Part 15B as a Class A digital device
- EN61326 Class A Electrical Equipment for measurement, control, and laboratory use — General Requirements

## FCC Warning and Notes

- Warning: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment
- Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his own expense
- Note regarding FCC compliance: Although this design of instrument has been tested and found to comply with Part 15, Subpart B, of the FCC Rules for a Class A digital device, please note that this compliance is voluntary, for the instrument qualifies as an "exempted device" under 47 CFR 15.103(c), in regard to the cited FCC regulations in effect at the time of manufacture
- Note regarding Canadian EMC compliance: Le présent appareil numérique n'émet pas de bruits radioélectrique dépassant les limites applicables aux appareils numériques de classe A prescrites dans le règlement sur le brouillage radioélectrique édicté par le Ministère des Communications du Canada

This Bio-Rad instrument is designed and certified to meet EN61010\* and the EMC requirements of EN61326 (for Class A) safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified or altered in any way. Alteration of this instrument will cause the following results:

- Void the manufacturer's warranty
- Void the EN61010 safety certification
- Create a potential safety hazard

\* EN61010 is an internationally accepted electrical safety standard for laboratory instruments.

Bio-Rad Laboratories is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad Laboratories or an authorized agent.

We strongly recommend that you follow the safety specifications listed in this section and throughout this manual. Use only the supplied power cord in the instrument, making sure to choose the plug adaptor that corresponds to the electrical outlets in your region.

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# **Chapter 1 – Introduction**



## Introduction

The Bio-Rad TC20™ automated cell counter is a benchtop instrument that counts mammalian cells in one simple step, initiating auto-counts immediately upon slide insertion. The innovative autofocus technology and sophisticated digital image analysis algorithm produce accurate cell counts for most samples in less than 30 seconds. The TC20 cell counter rapidly provides a total cell count with or without trypan blue staining and assesses cell viability via trypan blue exclusion. Getting all the data you need about your cell cultures is fast and easy; the TC20 cell counter and disposable counting slides eliminate the need for tedious setup, cleaning, or maintenance.

The TC20 cell counter provides accurate and reproducible cell counts for suspended cells that have been grown as adherent or suspension cells at concentrations within the  $5 \times 10^4$  to  $1 \times 10^7$  cells/ml range and for cells within the 6–50  $\mu\text{m}$  cell diameter range. Only 10  $\mu\text{l}$  of cell suspension is required for the cell count. The cell counter detects the presence of trypan blue in the sample with no user input required and automatically accounts for the 1:1 (trypan blue:cells) dilution factor. The TC20 cell counter can count single or mixed cell population samples. For samples with multiple cell populations the user can define the cell population of interest by adjusting the position of cell size gates.

An image of the counted cells can be viewed onscreen and downloaded onto a USB flash drive immediately after the count. The TC20 cell counter can store results from 100 counts. Count results can be exported onto a USB flash drive and viewed in Microsoft Excel. Each sample can be given a unique name and if the data autosave function is enabled, the JPEG image from the current count is automatically saved onto the inserted USB flash drive. Each image contains a metatag with the cell count data and can be further analyzed using the **TC20 Data Analyzer** downloadable from [www.bio-rad.com/tc20](http://www.bio-rad.com/tc20).

The TC20 cell counter has a dilution calculator to derive cell resuspension volumes required to achieve the cell concentration needed for the next experiment. A histogram of cell diameter distribution in the sample can be viewed after the cell count.

### Instrument Overview

The front panel of the TC20 automated cell counter (Figure 1) includes:

- **User interface** — a color digital screen and four keys for navigating the software
- **Slide port** — used to insert the cell counting slide

The side (right) panel of the TC10 automated cell counter (Figure 1) includes:

- **USB port A** (top port) — for connecting thermal printer or USB flash drive
- **USB port B** (bottom port) — for connecting thermal printer; the USB port B cannot be used for the USB flash drive
- **Power switch** — for turning the instrument on and off

The rear panel of the TC20 automated cell counter includes:

- **Power inlet** — used to connect the cell counter to an electrical outlet via the supplied power cord (select the appropriate plug adaptor for your region)



Fig. 1. TC20 cell counter.

## User Interface

The user interface contains a color LCD screen and the four navigation keys listed below:

- **Home** — for accessing the Home screen or for exiting any screen
- **Up and down** arrow keys — for moving to the desired setting within a screen
- **Enter** — for confirming a selection

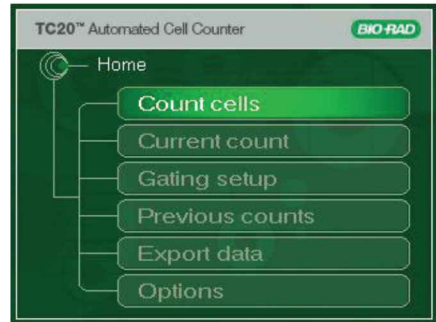


Fig. 2. Home screen.

## Home Screen

The Home screen can be accessed from any screen by pressing **Home**. This screen (Figure 2) provides access to the following choices:

**Count cells** — initiates a cell count. When this option is selected, the instrument will automatically start counting if a slide is already inserted.

If there is no slide in the instrument, the **Insert slide** screen will be displayed

**Current count** — for returning to the current count screen

**Gating setup** — for setting up histogram gating parameters

**Previous counts** — for viewing results from up to 100 previous counts stored in the instrument; results are organized by date/time stamps, with the most recent count shown first

**Export data** — for exporting results and image from the most recent count from the instrument onto a USB flash drive

**Options** — for selecting additional operations

### Options Screen

The Options screen (Figure 3) contains the following choices:

**Autosave/sample name** — for setting up the automatic export of cell images to a USB flash drive and for setting up sample naming preferences

**System test** — for verifying the performance of the instrument; a verification slide is needed for this function

**Set date/time** — for setting the date and time on the instrument, results are associated with date/time stamps

**Update firmware** — for upgrading the firmware

**Delete all counts** — for deleting all counts stored in the instrument

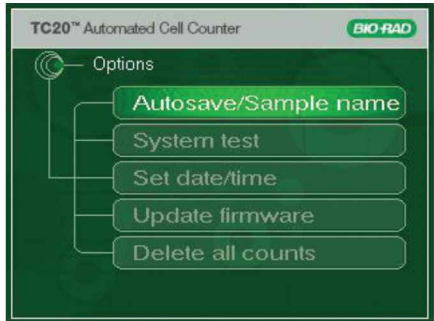


Fig. 3. Options screen.

### TC20 Automated Cell Counter Specifications

Processing time:	30 sec (may increase for higher concentration cell suspensions)
Cell concentration range:	$5 \times 10^4$ – $1 \times 10^7$ cells/ml
Optimal concentration range:	$1 \times 10^5$ – $5 \times 10^6$ cells/ml
Cell diameter range:	6–50 $\mu$ m
Dimensions (W x D x H):	19 x 15 x 25.4 cm
Weight:	2.2 kg

### Kits, Reagents, and Accessories

TC20 kits, reagents, and accessories are specially designed for use with the TC20 automated cell counter. These products are optimized to ensure accurate and reproducible cell counts.

### Counting Slides

The counting slide (Figure 4) is a disposable polygon plastic slide with a dual chamber composed of polymethyl methacrylate (PMMA). Each slide is 75 x 25 x 1.8 mm (W x D x H) with a chamber depth of 100  $\mu$ m. Each enclosed chamber requires only 10  $\mu$ l of suspended cells. The volume counted in each chamber is 0.4  $\mu$ l, equivalent to the volume counted in four squares (each 1 x 1 mm) of a manual hemocytometer.

The slide can provide counts for two different samples or duplicates of the same sample, one at a time. The chambers are labeled A and B, with an arrow next to each chamber to indicate the direction of slide insertion. Both slide chambers can be loaded prior to insertion in the TC20 cell counter for sequential counting.



Fig. 4. Counting slides and trypan blue dye.

### Trypan Blue Dye

The Trypan blue dye has been specially formulated for use with the TC20 counter to provide rapid staining for accurate determination of cell viability. The dye is composed of filter-sterilized 0.4% trypan blue dye (w/v) in 0.81% sodium chloride and 0.06% potassium phosphate dibasic solution.

Trypan blue is a vital stain recommended for use in estimating the number of live and dead cells in the cell suspension. The activity of this dye is based on the fact that the chromophore is negatively charged and does not react with the internal region of the cell unless the membrane is damaged. The dye enters into only dead cells and stains them blue, while live cells have intact cell membranes and remain bright and unstained.

### Thermal Label Printer

The thermal label printer (Figure 5) can be connected to the TC20 cell counter via the USB port A or USB port B. The printer, which uses a direct thermal printing method, provides labels with results that can be placed into a laboratory notebook.

### System Verification Kit

The verification kit (Figure 5) is available to verify the instrument's performance. The verification kit includes a verification slide and instructions for use.



Fig. 5. Accessories for the TC20 cell counter.

# 2

## **Chapter 2 – Setting Up the TC20™ Automated Cell Counter**

# Setting Up the TC20 Automated Cell Counter

## System Components

The TC20 automated cell counter (catalog #145-0102) includes:

- TC20 automated cell counter
- Power cord with four regional adaptors
- USB flash drive
- Instruction manual
- Quick guide
- Counting slides with dual chambers, 1 box of 30
- Trypan blue dye, 1.5 ml

Accessories that need to be purchased separately include:

- Label printer, 185 labels/roll
- Verification kit

## Unpacking the System Components

1. Unpack the TC20 automated cell counter carefully. Remove all packaging materials and store them for future use. Examine the instrument carefully for any damage incurred during transit. Ensure that all parts of the instrument including accessories listed above are included with the product. If any item is missing or damaged, contact your local Bio-Rad office.
2. Place the TC20 cell counter into upright position on a dry, leveled surface.
3. Insert the supplied power cord into the instrument.
4. Plug the power cord into the appropriate electrical outlet.
5. Turn on the instrument using the green power switch button. The **Home** screen appears.

## Setting the Date and Time

The TC20 cell counter uses a date/time stamp to track cell count results stored on the instrument. The date and time should be set on the TC20 cell counter before the first count. Resetting the date/time stamp after using the TC20 cell counter will not affect stored results.

1. Press the **down** arrow key to go to **Options** from the **Home** screen, and press **Enter**.
2. Use the **down** arrow key to go to **Set date/time**, then press **Enter**.
3. Use the **up** or **down** arrow key to move through the settings until you reach the setting you want to modify (for example, month) (Figure 6). The press **Enter** to find the correct option (for example September, October).
4. Use the **up** or **down** key to move to the next setting (for example, day) to be modified, and press **Enter** to find the correct option within the setting.

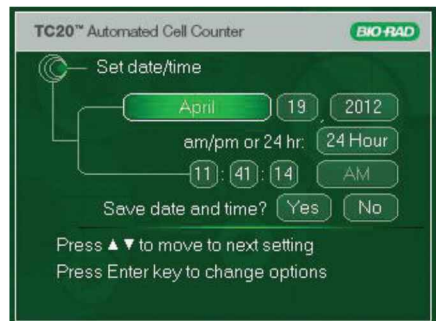


Fig. 6. Setting the date and time.

- Finally, when you have made all the modifications use the **up** or **down** arrow key to reach the **Yes** or **No** options for the **Save date and time?** setting.
- Press **Enter** to select **Yes** to save the changes and return to the Home screen or select **No** to return to the Home screen without saving the changes.

## Setting up Gating

TC20 cell counter has two counting modes, automated and user defined. In the automated mode (**User defined gates are Disabled**) the cell counting algorithm automatically identifies the cell population of interest. This mode does not require any user input and is suitable for immortal cell lines and samples that are composed of cells with similar cell size.

In the user defined mode (**User defined gates are Enabled**) the cell counter will count only objects within a user defined cell diameter range, this can be done by adjusting position of the low and high cell size gate (in  $\mu\text{m}$ ). Only objects within the range will be analyzed as cells, those with diameters outside of that size range will be excluded from the cell count.

Enable user defined gates when counting samples containing multiple cell populations with a **wide range of cell diameters** from which you need to select a population of interest, for example fibroblasts with lymphocyte background.

**User defined gates** — select **Enabled** option to switch to the user defined mode or select **Disabled** option to switch to the automated mode. Once enabled, a histogram showing all objects found in the sample will be displayed at the beginning of each count. Move the size gates to select the range of cell diameters the TC20 cell counter will include in the count.

**Use saved gates** — the **Yes** option will save the position of the size gates from the previous count for the next count, thus saving time when multiple sample replicates are counted as you may need to move the size gates on the pre-count histogram only a few microns or not at all.

If you select **No**, the size gates will flank what the TC20 cell counter presumes to be the population of interest.

**Image preview** — select **On** to display image of cells with diameters between the selected cell size gates. No image preview will be displayed if the **Off** option is selected.

To enable the **User defined** mode:

- Press the **down** arrow key to go to **Gating setup** (Figure 7) from the **Home** screen, and press **Enter**.
- Use the **up** or **down** arrow key to move through the settings (for example, **User defined gates**, **Image preview**) until you reach the setting you want to modify. Then press **Enter** until you find the correct option (for example, **Enabled**, **Disabled**).
- Use the **up** or **down** arrow key to move to the next setting to be modified, press **Enter** to find the correct option for the setting.

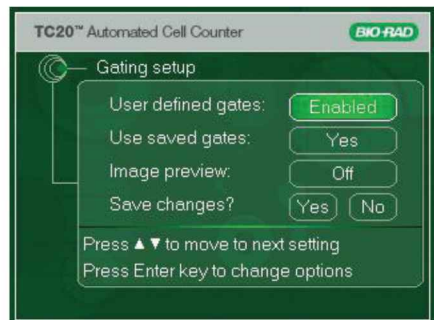


Fig. 7. Setting up gating.

## ■ Setting up the TC20 Automated Cell Counter

4. Finally, when you have made all the modifications use the **up** or **down** key to reach **Yes** or **No** options for the **Save changes?** setting.
5. Press **Enter** to select **Yes** to save the changes and return to the **Home** screen, or select **No** to return to the **Home** screen without saving the changes.

### Setting Up Automatic Data Export and Sample Naming

In this menu you can enable **automatic export of cell image** from the most recent count to a USB flash drive; the image can be viewed on your computer in JPEG format.

You can also use this menu to set sample naming preferences. Enabling **sample name** will allow you to select a name consisting of maximum eight alphanumeric characters. To save time the name selected for the most recent count will automatically be populated for the next cell count. You can then modify it, for example manually serialize it. When the automatic **name serialization** is enabled you can select a name consisting of maximum six characters, the last two characters are reserved for automatic numerical serialization. This unique sample name will be used as the JPEG file name and will be part of the result record listed in the **Previous counts**.

**Important:** At each new cell count the image from previous count is replaced by the image from the most recent count. When the instrument is switched off, the image from the last count is no longer kept in memory.

If the **Data autosave** is disabled, then the **Sample name** and **Serialize sample name** features are automatically disabled.

TC20 cell counter checks sample names already saved on the USB flash drive. If a sample name is already present on the flash drive, then you will be prompted to select another name. If a USB flash drive is not inserted, you will be prompted to insert it into USB port A.

To enable the sample naming and automatic data export:

1. Press the **down** arrow key to go to **Options** from the **Home** screen, and press **Enter**.
2. Press the **down** arrow key to go to **Autosave/sample name** (Figure 8), and press **Enter**.
3. Use the **up** or **down** arrow key to move through the settings (for example **Data autosave**) until you reach the setting you want to modify. Then press **Enter** until you find the correct option (for example, Enabled, Disabled).
4. Use the **up** or **down** arrow key to move to the next setting to be modified, press **Enter** to find the correct option for the setting.
5. Finally, when you have made all the modifications use the **up** or **down** key to reach **Yes** or **No** options for the **Save changes?** setting.
6. Press **Enter** to select **Yes** to save the changes and return to the **Home** screen, or select **No** to return to the **Home** screen without saving the changes.

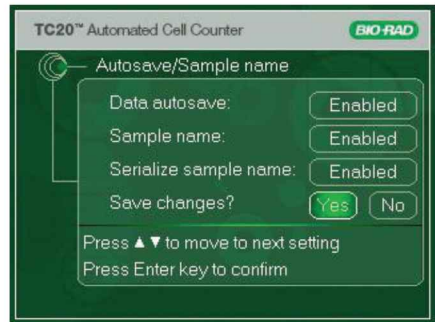


Fig. 8. Setting up data export and sample naming.



# 3

## Chapter 3 – Performing Cell Counts

# Performing Cell Counts

## Loading Slides

Handle the TC20™ counting slides using the edges and avoid touching the optical surface of the slides.

**Important:** When loading the sample, place the pipet tip at a 45° angle at the bottom of the sample loading area (half circle at outer end of the chambers). Slide the tip along the surface and carefully touch the apex of the half circle (Figure 9). Once the tip is stopped, depress the plunger to begin the capillary loading process. Care should be taken to avoid visible bubble formation or back splatter. Do not overfill or underfill the chamber. Overfilling the chamber and possible resulting accidental spillage of sample inside the instrument could lead to biological contamination of the cell counter.



Fig. 9. Loading the counting slide.

The cell counting slides cannot be reused. Dispose of used slides as biohazardous waste according to your local environmental health and safety regulations. To avoid injury do not break the counting slides.

## Preparing Samples

### Preparing Samples without Trypan Blue Dye

1. Pipet 10 µl of the cell suspension into the outer opening of either chamber of the counting slide (Figure 9).

### Preparing Samples with Trypan Blue

1. To determine cell viability, mix 1 part trypan blue dye and 1 part cell suspension: In a micro test tube or on Parafilm combine 10 µl of the cell suspension with 10 µl of trypan blue dye. Gently pipet up and down ten times to mix.
2. Pipet 10 µl of the mixture into the opening of either chamber on the counting slide.
3. When counting the sample in duplicate, combine 20 µl of the cell suspension with 20 µl of trypan blue dye, and then pipet 10 µl of the mixture into each chamber.

**Important:** The cell suspension must be loaded into the counting slides and counted immediately (within 5 minutes of mixing with trypan blue dye). Viable cells that are exposed to trypan blue dye for an extended period may start incorporating the dye, affecting the accuracy of the cell count.

Make sure the stock cell suspension is thoroughly mixed by pipetting or vortexing. Failure to do so can result in improper sample representation within the loaded counting chamber. Pipet the sample from the middle of the tube filled with stock cell suspension. Pipetting from the bottom or top can result in a sample with a higher or lower concentration, respectively.

If you work with adherent cells, use trypsin to get them into suspension. Using trypsin instead of scraping improves cell roundness and decreases the number of cell clusters.

## Counting Cells on the TC20 Automated Cell Counter

1. Simply inserting a counting slide into the slide slot of the TC20 cell counter will automatically initiate a cell count (Figure 10). The chambers are labeled A and B, with an arrow next to each chamber to indicate the direction of slide insertion. Make sure that the slide is completely inside the slide slot; otherwise the instrument does not detect the slide and counting does not start. Make sure the slide is inserted right side up.
2. The cell counter automatically detects the presence of the slide and initiates the count. The cell counter automatically detects the presence of trypan blue dye.
3. The count results appear on the **Current count** screen.

### For samples without trypan blue dye —

On the **Current count** screen, the instrument provides the total cell count per ml (Figure 11).

**For samples with trypan blue dye —** On the **Current count** screen, the instrument provides the total cell count per ml, live cell count per ml, and percentage of live cells (Figure 12). If the counted sample contains trypan blue, the instrument accounts for 1:1 dilution of trypan blue to cell suspension.

**Important:** If the number of cells is above or below the specified range of the TC20 automated cell counter, “Value out of range” is displayed on the **Current count** screen.

View the image of the cells (see **Viewing the Image of Cells from the Current Count** for details) and determine whether the sample should be diluted or concentrated. Then repeat the cell count.

If the result is between  $5 \times 10^4$  and  $5 \times 10^5$  cells/ml, counting multiple sample replicates will increase accuracy. After performing replicate measurements calculate the average and use it as your result. If the result is above  $5 \times 10^5$  cells/ml, only one measurement is needed.

4. Once the instrument completes the cell count, remove the slide from the slide slot. To exit the **Current count** screen, press **Home**.
5. If the slide is removed, results from the last count will remain on the screen until a new slide is inserted. When a new slide is inserted and the instrument starts counting, the previous result will be stored on the TC20 cell counter using a date/time stamp to track the count.



Fig. 10. Inserting the counting slide into the TC20 cell counter.

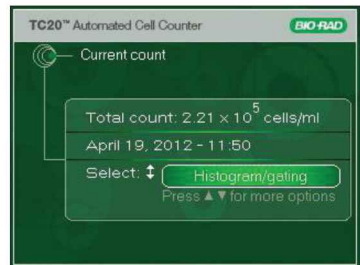


Fig. 11. Cell count screen without trypan blue dye.

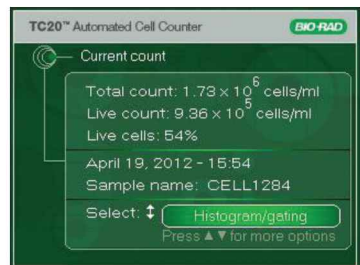


Fig. 12. Cell count screen with trypan blue dye.

## Counting Samples with Multiple Cell Populations

When counting samples with multiple cell populations that vary greatly in size (for example, fibroblasts with lymphocyte background) enable the **User defined gates** (Figure 7). A histogram (Figure 13) will be displayed at the beginning of the cell count; you can adjust the size gates to select the population of interest.

**Important:** This histogram displayed at the beginning of the count shows all objects present in the sample. After you have confirmed cell size range to be analyzed (delineated by position of the cell size gates) objects within that specified cell size range are analyzed as cells, those with diameters outside of that size range are excluded from the cell count.

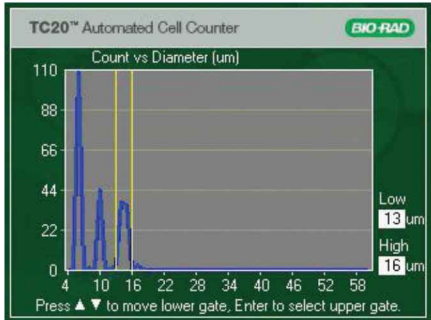


Fig. 13. Counting samples with multiple cell populations.

If **User defined gates** are **Disabled**, the TC20 cell counter will automatically (without input from the user) determine what the population of interest is.

If unsure which part of the histogram represents your population of interest, enable the manual mode and move the size gates to the extremes of the histogram. Then open the exported JPEG on your computer using the **TC20 data analyzer** and move the size gates until only the cells you are interested in are annotated in the image. Use the displayed size gate positions (in microns) as a guide for subsequent counts on the TC20 cell counter.

To select a population on the histogram displayed at the beginning of the cell count:

1. When the histogram is displayed the low size gate is flashing and positions of gates (in microns) are displayed in the lower right corner.
2. Use the **up** or **down** arrow keys to move the low gate to the desired position.
3. Press **Enter** to select the high gate. Use the **up** or **down** arrow key to move the high gate to the desired position.
4. Press **Enter** to confirm position of both gates.
5. Press **Enter** to proceed with the count, or press **Home** to start over. Press **Home** anytime during the process to start over.
6. Press **Home** twice to return to the **Home** screen.

## Sample Naming

When sample naming is enabled (see **Setting Up Automatic Data Export and Sample Naming** for details) you will be prompted to select a sample name for each cell count. You can select a name containing up to eight alphanumeric characters.

This unique sample name will be used as the JPEG file name and will be part of the result record listed in the **Previous counts**.

To save time, the sample name you selected for the previous count will automatically be populated for the next count and can be modified, for example manually serialized.

If automatic name serialization is enabled, you can select a name consisting of maximum six characters, the last two fields are reserved for automatic numerical serialization.

**Underscore symbol** — create a space in the sample name

**Dot (.)** — indicates the end of the name, cursor will move to the **Use name?** field

To name a sample:

1. Insert a counting slide into the slide slot of the TC20 cell counter to initiate a cell count.

2. At the end of the count you will be prompted to select a sample name (Figure 14). Use the **up** or **down** arrow key to scroll through the list of characters until you find the correct one. Then press **Enter** to confirm the selection.

3. Cursor then moves on to the next character field to be populated. Repeat this until you have selected all characters you need.

4. Cursor then moves onto the **Use name?** field. Select **Yes** to use the sample name, **Rename** to make modifications, or **No** to not use the

sample name (the default CELLxxx. will be used instead). Then press **Enter** to confirm.

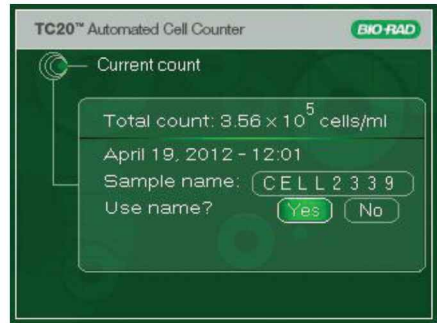


Fig. 14. Sample naming.

## Current Count Options

### Using the Dilution Calculator

The dilution calculator is used to calculate volume adjustments needed to achieve the cell concentration required for the next experiment. If trypan blue was used during the count, the instrument accounts for the 1:1 dilution with the dye in the calculation results, and only the live cell concentration will be used for calculations.

To use the recent count as the starting cell concentration, use the following instructions:

1. From the **Current count** screen, use the **down** arrow key to select **Dilution calculator**.
2. Press **Enter** to continue.
3. Use the **up** or **down** arrow key to select a value, and press **Enter** to confirm the selection. If the value of the desired cell count is higher than the starting cell count, "Invalid dilution" is displayed on the screen.
4. Repeat step 3 until all the parameters have been set.
5. To recalculate the dilution, continue pressing **Enter** to go back to the field to be modified. Subsequent changes to values automatically recalculate output results.
6. Once all the parameters have been set, the cursor lands on the **Print dilution results** field. To print the dilution results, use the **down** arrow key to select **Yes** and then press **Enter**.

## ■ Performing Cell Counts

### Printing the Current Cell Count

The TC20 thermal label printer can be connected to the cell counter for printing results from the most recent count. The printer, which uses a direct thermal printing method, provides labels with results that can be placed into a laboratory notebook.

To print results from the most recent count:

1. Connect the thermal printer using USB port A or USB port B on the cell counter.
2. From the **Current count** screen, use the **down** arrow key to select **Print count**.
3. Press **Enter** to start printing.

### Viewing the Image of Cells from the Current Count

To see the image from the most recent count:

1. Use the **down** arrow key to select **View image** from the **Current count** screen.
2. Press **Enter**.
3. Use the **up** arrow key to zoom in on an image and examine cells in detail. To zoom out, use the **down** arrow key (Figure 15).
4. To return to the **Current count** screen, press **Enter**.



Fig. 15. Image of cells.

### Exporting the Image of Cells from the Current Count

The image from the most recent count can be downloaded onto a USB flash drive and viewed on your computer in JPEG format and can be further analyzed using the TC20 data analyzer software. If the **Data autosave** is **Enabled**, the image from the most recent count is automatically exported to USB flash drive. If the **Data autosave** is **Disabled**, then the image can be exported directly from the current count screen or by using the **Export data** menu on the **Home** screen.

To export the image from the **Current count** screen:

1. Use the down arrow key to select **Export image** from the **Current count** screen, and press **Enter** to confirm.
2. If a USB flash drive is already inserted into USB port A, the export starts automatically. If there is no USB flash drive inserted into the instrument, "Insert USB flash drive into Port A to export current image. Press Enter to return" is displayed on the screen. Insert a USB flash drive into USB port A.
3. A bar showing the progress of the image download is displayed on the screen. When the image export is complete, "File saved as CELLxxx.JPG. Press Enter to return" is displayed.
4. To exit, press **Home**.

**Note:** If a new slide is inserted while the instrument is exporting an image, the instrument queues the count until it completes the export. The instrument then detects the slide and asks if it should start counting the sample in the new slide. If a new slide is inserted after the image is exported, the instrument starts counting the sample in the new slide.

### Viewing Histogram and Gating from the Current Count

The histogram of cell size distribution from the most recent count shows the approximate cell diameters (in  $\mu\text{m}$ ) on the X axis and the cell counts on the Y axis. The cell counts are based on the 4  $\text{mm}^2$  imaged area. For samples without trypan blue, the plot shows the total cell count (Figure 16). For samples with trypan blue, two plots are used to show the number of live and dead cells (Figure 17).

**Important:** The TC20 cell counter accurately counts samples with single or mixed cell populations. If you work with mixed cell population samples containing cells that vary greatly in size you should enable the **User defined gates** in the **Gating setup** menu. The histogram only shows approximate cell diameters; it is not intended to provide precise cell diameters.

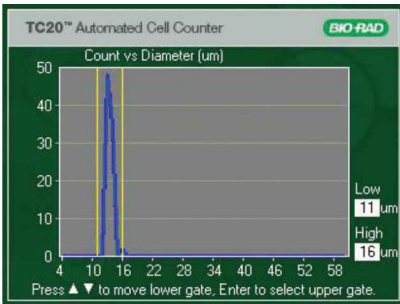


Fig. 16. Histogram for total cell count (sample without trypan blue dye).

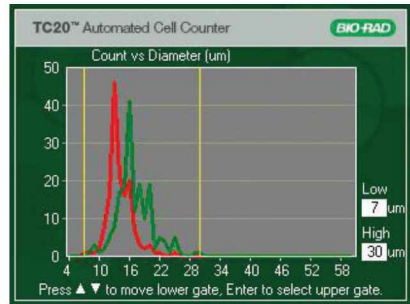


Fig. 17. Histogram for live and dead cell count (sample with trypan blue dye).

To gate the histogram from the most recent count:

1. Use the **down** arrow key to select **Histogram/gating** from the **Current count** screen, then press **Enter**.
2. When the histogram is displayed the low size gate is flashing and positions of gates are displayed in microns in the lower right corner.
3. Use the **up** or **down** arrow keys to move the low gate to the desired position.
4. Press **Enter** to select the high gate. Use the **up** or **down** arrow key to move the high gate to the desired position.
5. Press **Enter** to confirm position of both gates.
6. Gated cell count is displayed, press **Enter** to save it or press **Home** to start over. Press **Home** anytime during the process to start over.
7. Press **Enter** to return to the **Current count** screen.

# 4

## Chapter 4 – Saved Count Results



# Saved Count Results

## Viewing Previous Counts

The TC20™ cell counter stores results from 100 reads, with the first result shown being from the most recent count. When the instrument performs the 101st count, the oldest result is deleted from the internal memory. Results are stored by their date/time stamp corresponding to when the count results were generated.

To view previous count results:

1. From the **Home** screen, use the **down** arrow key to select **Previous counts**, and press **Enter**.
2. To scroll through the results slowly, use the **up** or **down** arrow key (Figure 18).
3. To scroll through results quickly, hold down the **up** or **down** arrow key.
4. To exit, press **Home**.

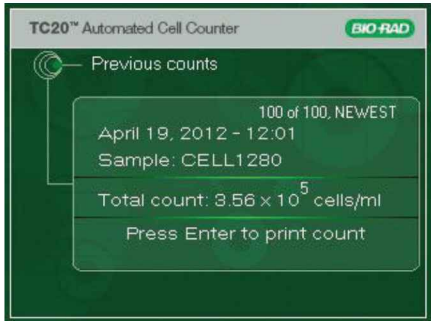


Fig. 18. Previous counts results.

## Printing Previous Counts

To print previous results:

1. Connect the thermal printer using USB port A or USB port B on the cell counter.
2. From the Home screen, use the **down** arrow key to select **Previous counts**, and press **Enter**.
3. Scroll through the results by pressing the **up** or **down** arrow key to find the result to be printed.
4. Press **Enter** to select **Print count**.
5. To exit, press **Home**.

## Exporting Data from Previous Counts

Data from previous counts can be exported to a USB flash drive from the **Export data** menu on the Home screen. Results from up to 100 previous counts can be exported and can be opened as a spreadsheet in Microsoft Excel. All results on the instrument will be exported. The JPEG image (with a metatag containing count data) from the most recent count can be exported as well; it can be opened on your computer and further analyzed using the TC20 data analyzer software.

To export all results from previous counts:

1. From the **Home** screen, use the **down** arrow key to select **Export data**, and press **Enter**.

2. Use the **down** key to select **Export previous counts**, and press **Enter**. If the USB flash drive is already inserted into instrument, the export starts automatically. Otherwise, "Insert USB flash drive into Port A to export counts. Press Enter to return" is displayed on the screen.
3. To proceed with the export, insert the USB flash drive into USB port A. The export starts automatically. Alternatively, cancel the data export by pressing **Enter**.
4. Once the previous counts have been exported, "File saved as RSLT.....TXT. Press Enter to return" is displayed.
5. To exit, press **Enter**.

To export image from the most recent count:

1. From the **Home** screen, use the **down** arrow key to select **Export data** and press **Enter**.
2. Use the **down** key to select **Export current image**, and press **Enter**. If the USB flash drive is already inserted into instrument, the export starts automatically. Otherwise, "Insert USB flash drive into Port A to export current image. Press Enter to return" is displayed on the screen.
3. To proceed with the export, insert the USB flash drive into USB port A. The export starts automatically. Alternatively, cancel the data export by pressing **Enter**.
4. Once the previous counts have been exported, "File saved as CELLxxxx.JPG. Press Enter to return" is displayed.
5. To exit, press **Enter**.

**Important:** At each new cell count the image from previous count is replaced by the image from the most recent count. When the instrument is switched off, the image from the last count is no longer kept in memory.

### Automatic Data Export

If the **Data autosave** is enabled (see **Setting Up Automatic Data Export and Sample Naming** for details) the image (containing metatag with count data) from the current count will automatically be exported to a USB flash drive inserted into USB port A on the cell counter. The image can be viewed on your computer in JPEG format and further analyzed with the TC20 data analyzer.

To automatically export image from current count:

1. Insert a counting slide into the slide slot of the TC20 cell counter to initiate a cell count.
2. If the USB drive is inserted, the image from that count will automatically be exported to the flash drive. If the USB flash drive is not inserted, "Insert USB flash drive into port A, Press Home to cancel" is displayed.
3. At that point either insert the flash drive or press **Home** to cancel the export. You can export the image after the count using the **Export data** option from the **Home** screen.

### **Deleting All Counts**

To delete all counts stored in the instrument:

1. From the **Home** screen, use the **down** arrow key to select **Options**, and press **Enter**.
2. From the **Options** screen, use the **down** arrow key to select **Delete all counts**, and press **Enter**.
3. "Delete all counts?" appears on the screen.
4. Use the **up** or **down** arrow key to select **Yes** or **No**, press **Enter** to confirm.
5. The **Home** screen appears.

# 5

## **Chapter 5 – Maintenance and Troubleshooting**

# Maintenance and Troubleshooting

## Performing the TC20 System Verification Test

The TC20™ automated cell counter is calibrated at the time of manufacture and does not require further adjustment by the customer. A system verification slide can be purchased to validate the functionality and performance of the TC20 cell counter. It is a printed glass slide with a defined number of circles printed on each side.

To perform the TC20 system test:

1. Place the TC20 cell counter on a dry, level surface.
2. Insert the supplied power cord into the instrument.
3. Plug the power cord into the appropriate electrical outlet.
4. Turn on the instrument using the green power switch.
5. When performing the system test, **User defined gates** must be disabled. From the Home screen arrow down to **Gating setup**; press **Enter**.
6. Arrow down to **User defined gates** and press **Enter** until **Disabled** is selected (Figure 19).
7. Arrow down to **Yes** in the Save changes setting and press **Enter**. The Home screen appears.
8. Arrow down to **Options** and press **Enter**.
9. From the Options screen, arrow down to **System test**; press **Enter**.
10. When prompted, insert side A of the verification slide into the slide port. Press **Enter**.
11. When prompted, remove the slide from the instrument and insert side B of the slide into the port. Press **Enter**.
12. Pull out the verification slide and press **Enter**. This checks for debris on the optics.
13. The results of the system test are displayed on the System test screen (Figure 20). For each sample, a Pass or Fail result is reported, along with the number of objects found and expected, and the ratio of live objects (example: Pass 218 of 221, 0.50). The number expected can change each time the test is repeated, due to slight variations in slide position.
14. System test results can be exported via USB using the **Export previous counts** function. (See Exporting Data from Previous Counts for details.)
15. Save the verification slide for future use.



Fig. 19. Disabling user defined gates.

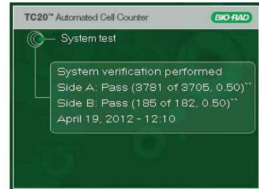


Fig. 20. System verification results.

**Important:** If the cell counter fails the system test procedure, a reason for the failure will be displayed on the System test screen. The TC20 counter will indicate either cleaning the verification slide and repeating the test or contacting Bio-Rad Technical Support.

Do not insert the verification slide if it is damaged. It could damage the instrument.

Testing the system once a month is recommended but can be done as often as daily. Neither test frequency will modify the slide in any way.

## Cleaning the TC20 Automated Cell Counter

The TC20 cell counter requires little maintenance but with long and constant use it requires some cleaning and other maintenance. The instrument should be cleaned on a regular schedule to remove debris or dirt that might interfere with proper function.

**Cleaning the case** — always turn off the instrument and disconnect the power cable before cleaning the case. Wipe the outer case with a soft, lint-free cloth and deionized water. Avoid wetting the power switch or jack.

**Warning!** Never pour or spray water or other solutions directly on the instrument. Wet components can cause electrical shock when the cell counter is plugged in.

**Cleaning the LCD screen** — always turn off the instrument and disconnect the power cable before cleaning the LCD screen. Wipe the screen with a soft, lint-free cloth lightly moistened with LCD cleansing detergent. Excessive force can damage the screen. Wipe the screen dry immediately.

**Warning!** Do not use abrasive detergents or rough material, as they may scratch the control panel and display.

**Decontaminating the instrument** — always turn off the instrument and disconnect the power cable before decontaminating the unit. Wipe the outer case with a soft, lint-free cloth and 70% alcohol.

**Warning!** If you use a 10% bleach solution it may leave a residue of bleach crystals that over time may scratch the surface. Avoid touching or cleaning the LCD screen and control panel. Follow by wiping down with a damp cloth to remove any excess bleach.

Do not spill liquids inside the cell counter, for example by overfilling the slides or tipping the cell counter over while a slide with sample is in the sample port. This could contaminate the internal structure of the unit and would require professional decontamination.

## Thermal Printer Maintenance

The print head is the most critical component of a printer and should be cleaned every time you start a new label roll. The print head is composed of metallic heating elements located inside the printer, in front of the yellow triangle heat indicator.

To clean the print head:

1. Turn off the printer and disconnect the power connector.
2. Open the printer and remove the label roll.
3. Wet a white cotton cloth (or a cotton swab) with 99% isopropyl alcohol and wipe off the label adhesive residue and paper dust from print head heating elements.

**Loading a new roll of labels into the printer** — in order for the printer to cut labels correctly it must calculate the size of both the label and the gap between labels. It can do this only if the new roll is already loaded when the printer is powered on.

Loading a new roll with the printer off leads to inaccurate label cutting and adhesive buildup on the print head.

## ■ Maintenance and Troubleshooting

To load a new roll of labels:

1. Turn the printer on.
2. Open the cover.
3. Place the new roll as directed, with the first label protruding approximately 1.5 cm.
4. Close the printer cover. Printer is now ready for use.

**Printer is powered off with labels already installed** — if the printer is powered off with media already installed, perform the following procedure:

1. Turn the printer on.
2. Open the cover.
3. Pull the first label so it protrudes approximately 1.5 cm.
4. Close the printer cover. Printer is now ready for use.

### Updating the Firmware

The firmware version installed in the TC20 automated cell counter is displayed when the instrument is switched on.

To update the firmware on the instrument:

1. Insert a USB flash drive with the firmware file into USB port A. The firmware file must be saved in the root directory of the USB flash drive.
2. From the Home screen, arrow down to **Options** and press **Enter**.
3. From the Options screen, arrow down to **Update firmware** and press **Enter**.
4. When prompted, insert the USB flash drive containing the firmware update into USB port A. “Validating” appears on the screen.

**Note:** If the USB flash drive does not contain a firmware update or if the update file is not saved in the root directory, “No update file present. Press Home to exit” is displayed.

5. When the instrument recognizes and validates the firmware update, it displays the current and new firmware version numbers and “Press Enter to continue updating”.
6. Press **Enter** to continue updating.
7. When the update is successfully completed, the TC20 cell counter automatically restarts.

### Battery

The TC20 cell counter uses a 3 V lithium coin cell battery with a 10-year expected lifetime to maintain the date setting. If the date changes without user input, it may indicate that the battery is getting weak. Contact Bio-Rad technical support for assistance with battery replacement.

**Warning!** Do not attempt to change the battery. Contact the Bio-Rad technical support team.

#### For the State of California, USA only.

Perchlorate material — special handling may apply.

See [www.dtsc.ca.gov/hazardouswaste/perchlorate](http://www.dtsc.ca.gov/hazardouswaste/perchlorate) for more information.

Perchlorate material: lithium battery contains perchlorate.

## Troubleshooting

Error Message	Possible Cause and Solution
Invalid result data	Data were improperly stored and cannot be retrieved.
Invalid dilution	The value entered for the desired cell concentration is higher than the starting cell concentration. Ensure that the value of the desired cell concentration is not higher than the starting cell concentration.
Incorrect slide, remove slide. Insert verification slide, side A first, press Enter	The counting slide is used instead of the verification slide during system validation. Insert side A of the verification slide, and press <b>Enter</b> .
File write error Press Home key to continue No disk found Disk is full	<ol style="list-style-type: none"> <li>1. Check USB flash drive. If it is close to full, then empty it.</li> <li>2. If "File write error" is displayed after step 1, then reformat flash drive in FAT32 format.</li> <li>3. If "File write error" is displayed after step 1 and step 2, then use different flash drive.</li> </ol>
No firmware update file present, press Home key to exit	The USB flash drive does not contain the updated firmware file or the file is saved outside of root directory of UBS flash drive. Insert a USB flash drive that contains the updated firmware file in the root directory.
Please repeat the test with a clean slide	Failure of system test. Repeat the verification without the verification slide. If the problem persists, contact Bio-Rad Technical Support team (see <b>Bio-Rad Laboratories Resources</b> for details).
Slide removed during focusing. Reinsert slide to continue or press Home to exit	The slide is removed during counting. If the slide is removed during focusing, counting is interrupted. Once the slide is reinserted, the count starts automatically.
Connect the printer	The thermal label printer is not connected to the instrument. Connect the printer to the instrument via the USB port A or B.
No labels in the printer	There are no labels in the thermal label printer. Insert labels into the printer.
Current count value out of range	The number of cells is above or below specification. View the cell image and dilute or concentrate your sample. Repeat the count.
Exposure time too high	Slide is too dark; remove it and use a clean counting slide.
Printer error	Bad status code returned for the printer. Check USB cable connecting to the printer. Turn the printer off, then turn it back on.
Filename invalid	Bad sample name entered. Re-enter a valid sample name.
Store results failed	Previous count data storage failed. FLASH memory corrupted. Export any good previous counts, then delete all counts (reformat FLASH).
Hardware errors (stepper motor error, bright field LED/CMOS not working, SPI communication not working, vinculum communication error, CPLD, FPGA)	Restart the instrument; if the error is displayed again, contact Bio-Rad technical support team (see <b>Bio-Rad Laboratories Resources</b> for details).



## Ordering Information

Catalog #      Description

### TC10 Automated Cell Counter

145-0102      **TC20 Automated Cell Counter**, 120–240 V, includes instrument, power supply, USB key, USB cable, 30 double chamber counting slides, 1.5 ml trypan blue dye

145-0103      **TC20 Automated Cell Counter with Label Printer**, 120–240 V, includes instrument, power supply, USB key, label printer, 1 roll of labels, USB cable, 30 double chamber counting slides, 1.5 ml trypan blue dye

### Kits and Reagents

145-0003      **Counting Kit**, includes 30 double chamber counting slides (60 count), 1.5 ml trypan blue dye

145-0014      **System Test Kit**, includes verification slide, instructions

145-0021      **Trypan Blue Dye 0.4%**, includes 5 x 1.5 ml trypan blue dye (750 count)

145-0022      **Trypan Blue Dye 0.4%**, includes 10 x 1.5 ml trypan blue dye (1,500 count)

### Accessories

145-0005      **Label Printer**, 120–240 V, includes label printer, USB cable, 1 roll of labels

145-0007      **Printer Labels**, for label printer, 500 labels/roll

145-0015      **Counting Slides**, includes 150 double chamber counting slides (300 count)

145-0016      **Counting Slides**, includes 300 double chamber counting slides (600 count)

145-0017      **Counting Slides**, includes 600 double chamber counting slides (1,200 count)

145-0018      **Counting Slides**, includes 900 double chamber counting slides (1,800 count)

145-0019      **Counting Slides**, includes 1,200 double chamber counting slides (2,400 count)

145-0020      **Counting Slides**, includes 2,400 double chamber counting slides (4,800 count)



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