ADAM SCC A New Standard of Automatic Cell Counter



INSTRUCTION MANUAL

NESMU-ASC-001E (V.1.0)



Developed and Manufactured by NanoEnTek Inc.

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ADAM-SCC, User's Manual

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The information in this manual is described as correctly as possible and is applicable to the latest firmware and software versions, but it may be changed without prior consent or notification.

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Safety Precautions

- 1. Always ensure that the power supply input voltage match the voltage available in your location.
- 2. For operation environment, See "Appendix C Technical Specification".
- 3. This machine is air-cooled so its surfaces become hot during operation. When installing it, leave a spaces of more than 10 cm (4 inches) around it.
- 4. Never insert metallic objects into the air vents of the instrument as this could result in electrical shock, personal injury and equipment damage.
- 5. Always set the main switch on the power supply unit to " \bigcirc " (OFF) before connecting the power cord to the wall outlet.
- 6. Always ensure that the grounding terminal of the instrument and that of the wall outlet are properly connected. The power cord should be connected to a grounded, 3-conductor power outlet.
- 7. To avoid potential shock hazard, make sure that the power cord is properly grounded.
- 8. Do not position the equipment so that it is difficult to operate the disconnecting device.
- 9. Be sure to set the main switch to " \bigcirc " (OFF), unplug the power cord and lock the stage before moving.
- 10. If the instrument is broken or dropped, disconnect the cord and contact a authorized service person. Do not disassemble the instrument.
- 11. Use only authorized accessories.
- 12. Use this equipment only as specified in this manual and as specified in any documentation associated with its components. Any use of the equipment in an unspecified manner is strongly discouraged and may result in damage or injury as cautioned by signed warnings.



Safety Symbols

The following symbols are found on the instrument and this document. Study the meaning of the symbols and always use the equipment in the safest possible manner.

Symbol	Meaning			
\wedge	Caution & Warning			
	ON (Power)			
\bigcirc	OFF (Power)			
	Protective earth (Ground)			
CE	This instrument and consumables conforms to the Declaration of Conformity.			
À	Caution, Biohazard Protective measures must be used in dealing with biologically hazardous materials such as carcinogenic reagents.			



Warnings

Item	Warning	Date
Battery inside device	Risk of explosion if battery is replaced by an incorrect type. This battery is not replaceable by a user. Refer to a qualified personnel.	Aug 01, 2008
Cover	Do not remove a cover or dissemble a case. There is no adjustable components inside the instrument. If malfunction is found, refer to a service personnel.	Aug 01, 2008
Manual	Do not attempt to service the equipment unless this manual has been consulted and is understood. This manual is available in English only. Failure to heed this warning may result in injury to service provider, operator from electric shock, mechanical or other hazards.	Aug 01, 2008
Sample handling	Wear gloves during sampling. User's sample may have the infectious biohazardous substance.	Aug 01, 2008
Waste	After using Accuchips, appropriately dispose it as biohazardous waste. Do not reuse the Accuchips.	Aug 01, 2008
Operator	Must have the general knowledge of cell counting procedure and bio safety to handle the sample that may have the infectious biohazardous substance	Aug 01, 2008



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1-1. Technology - Mechanical

Until now, cell counting and viability measurement for many types of cells have been performed manually using hemocytometer with Trypan Blue exclusion method which is to distinguish viable cells from non-viable cells. One drawback of this method, however, is the propensity for the staining of artifacts; another drawback is that the naked eye can only differentiate between cells in a limited concentration range in the hemocytometer chamber. This combined with the potential problem of cell aggregation and limited sample volume leads to the common variation of counts normally associated with this method.

To address these problems, Digital Bio has developed the ADAM, which is based on a fluorescent microscopy technique for counting cells. The ADAM utilizes sensitive fluorescence dye staining, LED optics and CCD detection technologies to make the cell analysis more accurate and reliable.

To count cells using ADAM, the cells are mixed with a Propidium Iodide (PI) stain and directly pipetted on to a disposable plastic chip. The chip is then loaded onto a precision stage. An ADAM system is automatically focused onto the chip and cells that have been stained are recorded by a sensitive CCD camera. The image results are automatically processed generating the cell count which is displayed on the front of the instrument. Simple. Fast. Accurate. Reliable.



Section 1. Introduction

1-2. Technology - Measurement Section

SCC Kit (CRS-K01) is composed of Propidium iodide (PI) for counting somatic cells. CRS-K01 can be used without diluting raw milk.

Measuring range of cell density is 0 to 1×10^7 cells/ml.

One kit is capable of 100 tests. Each tube has 100 μ l reagent of somatic stain solution. Simply add the same volume of the raw milk sample in the tube then every preparing for experiment end. Once the experiment is complete the results can be printed through the thermal print. Printed number indicates cell concentration (\times 1000/ml) in each channel.

- T: The cell concentration in the T channel of the chip
- N: The cell concentration in the N channel of the chip

Store kit box upright and at room temperature. Expiration date of stain solution is written on the bottom of the kit box (yy-mm-dd). Be sure to check the expiration date before using. Follow the exact steps detailed in the Instructions for Use section.

Add 100 μ l of the raw milk sample in the tube.

Mix the sample thoroughly by turning the vial upside down 3-5 times. Load 20 μl of the cell sample onto the Chip. Ensure that no bubbles enter the channel.



2-1. Packing List

The ADAM is shipped with the following components. Once you receive your instrument, please check that all items listed below were shipped. If any items are missing or damaged, contact your local distributor or sales@digital-bio.com.

Item	Quantity
Main device	1
Somatic cell counting SCC Kit (CRS-K01)	1
Instruction Manual	1
External video monitor (Optional)	1
Installation CD	1
Key Pad	1
Power Cord	1
USB cable	1
Fuse	2

After receiving ADAM, examine it carefully for any damage incurred during transit. Any damage claims must be filed with the carrier.



CAUTION:

Neglecting to remove any or all shipping brackets or foams prior to operation may result in damage to the equipment The shipping brackets or foam inserts must be reinstalled prior to shipping the unit to prevent damage to the equipment.



2-2. Identification of System Components

Fig. 1. View of the ADAM



Key Pad

- 1. Control buttons:
- ▲ Eject: Ejects the chip holder from the Adam.
- Run/Start: Performs all procedures of automatic counting
- Parking: Protects the alignment of stage from external shock when the ADAM is moved to the other places.
 - For It is strongly recommended to park ADAM before turning it off.
- 2. <u>Door:</u> Chip holder comes out here.
- 3. <u>LCD</u>: Displays the process and the result.
- 4. <u>Keypad:</u> Inputs the sample number and "Enter" button . Less than 3 characters.
- 5. <u>External video monitor</u>: To see the actual cell shape and check if any clumped cell through this monitor.



2-3. Identification of System Components - continued

Fig. 2. Rear view of the ADAM



- 1. Fan: Adam's cooling fan
- 2. Power switch: Main power on/off control.
- 3. Power plug: Connect the ADAM power cord to wall outlet
- 4. Video Port: External video monitor port
- 5. <u>USB port:</u> Connect the USB serial cable to computer
- 6. RS-232C serial port: Not Connected(Port for only QC and Service)
- 7. Key pad port: Connect the Keypad



3-1. Environmental Requirements

To insure correct operation and stable performance over an extended period of time, install the ADAM in a location which meets the following conditions:

- Room temperature between 20 and 35 °C. Not recommended for cold room use (4 °C). <u>CAUTION: At low temperature (≤10°C), please</u> warming up the system for 10 min.
- Not exposed to direct sun light.
- Not subject to direct or continuous vibration.
- Not subject to intense magnetic or electromagnetic fields.
- Relative humidity between 0-95%.
- Area free from corrosive gases or other corrosive substances.
- Area with very little dust or other airborne particles.
- Allow a 10 cm minimum space around the instrument for proper air flow.
- Not allow to put heavy material on top of ADAM

3-2. Power on and Initial Display

- 1) Check the connection of the main device power cord.
- 2) Make sure that the main power switch is in the "I" (ON) position. (On the rear side of the main device.)

• When you turn on the ADAM, it will go through self diagnostic tests including, all optical components. If a problem is detected, please contact your local distributor or sales@digital-bio.com. If boot up is successful and no errors are detected, the home screens will be displayed as below.





3-3. Icon Functions







4-1. Material & Method

The following list contains the items needed for somatic cell counting using the Adam.

A raw milk sample, SCC Kit, Pipette and tips

1) The prepared materials ready to be used



2) Add 100 μl of the raw milk sample in tube.



3) Mix the sample by turning the vial upside down 3-5 times.



4) Load the cell sample onto the Chip. Ensure that no bubbles enter the channel.





4-2. Operating the ADAM

- 1) Press " EJECT" button on the main device to eject the chip holder.
- 2) Insert the Chip loaded with the sample onto the chip holder. Please be careful not to make bubbles.
- 3) Press the "▶, Run" button on the main device.
- 4) Automatic Focus will be carried out at the first time the device is booted. Once ADAM have done the Auto Focus process and on the following time, focusing process will be skipped.
- 5) After calculating the cell number, the chip will be ejected automatically. Then chip can be removed.
- 6) The calculated cell number per 1ml will be displayed automatically.
- 7) For another experiment, repeat the process from steps $1 \sim 5$.
- 8) Operator should remember the above procedure to perform the automatic cell counting with ADAM





4.3 Menu setting

You can set the menu as you press the *' button on the keypad from the screen for inputting cow numbers.



You can find the setting modes by selecting the number from the Menu.

4.3.1 Chip Selection

User can select the 2 kinds of chip type. One is the two channel chip (AccuChip^{2X}). Another is the four channel chip (AccuChip^{4X}).



After pressing the 'Enter' key, the screen will return to the Menu screen automatically.



Section 4. General Operation

4.3.2 LCD contrast

Press the number 4 key to adjust the brightness of the LCD screen from the MENU.



Adjust LCD contrast if the letters do not appear clearly on the screen. Press the 'Enter' key after you input the three digits. The range is from 600 to 750. After pressing the 'Enter' key, the screen will return to the Menu screen automatically.

4.3.3 System Information

igital Bio

The device versions and date which have been installed in the device can appear when the number 5 key is selected from the MENU. After pressing the 'Enter' key, the screen will return to the Menu screen automatically.



Section 4. General Operation

Press the `*' key after menu setting. Once inputted, the screen will return to the counting mode automatically.

4-4. Result Analysis

	12 : 00		12 :00
ID	x1000 /mL	ID	x1000 /mL
T:123	38	T1:123 N1:45	24 586
N:456	124	T2 : 678 N2 : 789	40 135

If the density of sample is over-range, you may see "Check Sample" or "Over Range" message at display monitor.



If you see those messages, please check below list first.



- < In case of "Check sample!" >
 - 1) Check sample contamination, or Chip with dust or other materials.
 - 2) Check mixture of sample and agent. It has to be mixed well.
 - 3) Check test tip whether stained with something.

If you have problems that mentioned above, you will get a result sheet like below.

Number	Date	Time	Cow	Count (SCC*1000/mL)	Chip	Channel	
0012	2010/03/23	21:50:31	12	87	4 CH	N2	
0011	2010/03/23	21:50:31	11	25	4 CH	Т2	
0010	2010/03/23	21:50:31	10	S - E	4 CH	N1	
0009	2010/03/23	21:50:31	9	65	4 CH	Τ1	<u> </u>
0008	2010/03/24	03:51:56	8	0	4 CH	N2	
0007	2010/03/24	03:51:56	7	5	4 CH	Т2	
0006	2010/03/24	03:51:56	6	13	4 CH	N1	
0005	2010/03/24	03:51:56	5	0	4 CH	Τ1	
0004	2010/03/24	03:39:17	4	54	4 CH	N2	
			-				

- < In case of "Over Range!" >
- 1) In case of the result of cell-counting is over 4000[X1000mL]
- 2) Check mixture of sample and agent. It has to be mixed well.
- If you have problems that mentioned above, you will get a result sheet like below.

Number	Date	Time	Cow	Count (SCC*1000/mL)	Chip	Channel	
0012	2010/03/23	21:50:31	12	87	4 CH	N2	
0011	2010/03/23	21:50:31	11	25	4 CH	Т2	Ш
0010	2010/03/23	21:50:31	10	R-0	4 CH	N1	
0009	2010/03/23	21:50:31	9	65	4 CH	Τ1	
0008	2010/03/24	03:51:56	8	0	4 CH	N2	
0007	2010/03/24	03:51:56	7	5	4 CH	Т2	
0006	2010/03/24	03:51:56	6	13	4 CH	N1	
0005	2010/03/24	03:51:56	5	0	4 CH	Τ1	
0004	2010/03/24	03:39:17	4	54	4 CH	N2	
0003	2010/03/24	03:39:17	3	57	4 CH	Т2	



4-5. Maintenance and Cleaning

ADAM does not need regular maintenance.

ADAM has no replacement of consumable materials

Clean the exposed outer surface of ADAM using a soft cloth and isopropyl alcohol or deionizes water.



CAUTION:

Dispose of wipes in an appropriately labelled solvent contaminated waste container.



5-1. Connection between ADAM and computer

The following steps will guide you to connect USB cable.

- 1) Connect the USB cable to ADAM.
- 2) Connect the USB cable to Desktop or Laptop computer.
- 3) Turn on ADAM and Desktop computer.





5-2. ADAM Report Software Installation

To install the Adam Report software, follow the directions as below.

- Insert the installation CD-ROM into the computer. Then open the file "Setup_ADAM_v1.x.x.exe". (Report program can be installed in Windows 2000, XP or higher version.) The start-up dialogue of the software, as shown below, will appear. Click "Next" to start installation.
- 2) If you want to change installation folder, click "Browse" and choose the location that you want. After choosing installation folder, click "Install" to proceed with the installation. The computer activates the "Installation of the Software".
 - Initial installation folder is "C:\Program Files\Digital Bio\ADAM".
- 3) Report Program will be installed automatically.
- 4) Click "Ok" to finish the installation.
 - * If the installation was successful, the report program can be found at **Start>All Program>ADAM**.





6-1. ADAM Report Program: Introduction

This Report Program is designed to manage and report all results from ADAM. All measurement results are automatically saved on the memory of ADAM. The user can download the data from the memory of ADAM and export it to Excel (*.xls) format. The user can delete data from memory of ADAM or can save captured images into Desktop or Laptop hard drive. The data list window consists of the sample number, chip, date, time, total, nonviable, viable, viability counting result in %.

• <u>CAUTION</u>: Before running the program, check the connection of USB cable between the Adam and the laptop or desktop computer.

						Ima	ige		
								Start	Park Ej
									Read data
								Auto Save	Delete data
								OFF ON	Export Exce
								Image path	
								C:₩Temp₩1	B
Number 0	Date	Time	Cow	Count (SCC*1000/mL)	Chip	Channel	Volume	0.132412	
0010 2010	0/03/23 :	17:32:19		10 0	4 CH		Total de	ad pixel : 00000000	0
0009 2010	D/03/23 :	17:32:19			4 CH	Т2			
0008 2010	D/03/23 :	17:32:19		1006	4 CH				
0007 2010	0/03/23	17:32:19		1776	4 CH	T1			
0005 2010	0/03/23	17:23:32		100	4 CH	N2 T2			
0003 2010	0/03/23	17:23:32		2013	4 CH	N1			
0003 2010	0/03/23	17:23:32		1776	4 CH	T1			
0002 2010	0/03/23	17:08:10							
0001 2010	0/03/23	17:08:10		642	2 CH				

Fig1. Main Frame of ADAM Report Program



Section 6 ADAM Report Program Guide

6-2. ADAM Report Program: Function Guide



- ① Image frame Image captured by ADAM will be shown here
- ② Function Buttons Start cell counting, saving images, exporting data, and all function of Report Program are handled by using these buttons (see p.24 for more detailed information of each button)
- ③ Data List All saved data in ADAM will be loaded and shown in data list section
- ④ Information Operation and counting results of each frame will be displayed here
- ⑤ Graph Analysis of results including cell size and frame by frame counting will be shown in graph section



② Function Buttons



Start cell counting



Park (Lock) stage of ADAM



Eject chip holder out of ADAM



Loads the experiment data from the memory of the main device.

Delete data Deletes all of the loaded data and memory of the main device.



Transfers the data list to Excel format and saves it.



Turn on or off automatic image save option

Default image save folder is "C:\Program Files\Digital Bio\ADAM\Images". Images will be saved until your hard drive has no more capacity to save. Be sure to set Auto Save off, unless you need to save images.

Example of saved image file: 081221(yymmdd)-203482(hhmmss)-N1(channel name)-002.bmp

Browse Choose folder to save images automatically



③ Data List

Number	Date	Time	Cow	Count (SCC*1000/mL)	Chip	Channel	
0012	2010/03/23	21:50:31	12	87	4 CH	N2	
0011	2010/03/23	21:50:31	11	25	4 CH	Т2	
0010	2010/03/23	21:50:31	10	105	4 CH	N1	
0009	2010/03/23	21:50:31	9	65	4 CH	Τ1	
0008	2010/03/24	03:51:56	8	0	4 CH	N2	
0007	2010/03/24	03:51:56	7	5	4 CH	Т2	
0006	2010/03/24	03:51:56	6	13	4 CH	N1	
0005	2010/03/24	03:51:56	5	0	4 CH	Τ1	
0004	2010/03/24	03:39:17	4	54	4 CH	N2	
0003	2010/03/24	03:39:17	3	57	4 CH	Т2	
0002	2010/03/24	03:39:17	2	13	4 CH	N1	
0001	2010/03/24	03:39:17	1	0	4 CH	Τ1	
							Н
							•
•						•	

Data list shows data stored in ADAM memory. Total amount of stored results are indicated at bottom of list as "Saved experiment". Up to 1000 counting results are automatically saved to ADAM memory. When memory of ADAM is full, new counting result will replace old data. These data can be exported as Excel Sheet (*.xls) and stored in personal computer or can be erased from ADAM memory.

④ Information

T1 count : 12 - 68 T1 count : 13 - 71 T1 count : 14 - 69
T1 count : 15 - 79
T1 count : 16 - 83
T1 count : 17 - 83
T1 count : 18 - 75
T1 count : 19 - 76
T1 count : 20 - 78
T1 count : 21 - 71
T1 count : 22 - 72
T1 Auto Count : 1030504
T1 error frame : 0
T1 Total : 1604
N1 count : 1 - 95
N1 count : 2 - 64
N1 count : 3 - 64
N1 count : 4 - 92
N1 count : 5 - 69
N1 count : 6 - 91

This section shows information regarding operation of ADAM. If cell counting is started through Report Program, the counting results of each frame that ADAM captures will be shown here.



Section 6 ADAM Report Program Guide

5 Graph



This section shows information of cell size distribution and counting results of each frame that ADAM captured. Through cell size graph, you can figure out whether there are cell clumps or aggregates. In case of counting evenly distributed cells without any aggregation, there should be a single peak on distribution of cell size.

 The size of cell in graph is not real size of cell. It is the size of pixels in fluorescence image captured by ADAM. And the size information is only to judge if there is a lot of aggregated cells.



Troubleshooting Table

Problem	Cause	Solution	
ADAM does not power up	Power switch in off position. No power from outlet. Bad power cord.	 Check power switch on back of unit. Check power source. Replace. 	
Inaccurate result	Cell number may be out of range. SCC kit solution has expired.	 Check the expired data. Try again after vortexing the cells 	
Software does not work	PC setup incorrect/wrong instruct mode. Cable's not fully connected/ wrong adaptor.	 Check program setup. Check all connections. 	
When error message is shown (& For information on each error message, see	When there are too many frames with errors (Error message: "Check sample!)	 Check sample contamination, or Chip with dust or other materials. Check mixture of sample and agent. It has to be mixed well. Check test tip whether stained with something. 	
page 20.)	When too many cells are loaded (Error message: "Over Range!")	 In case of the result of cell- counting is over 4000[X1000mL] Check mixture of sample and agent. It has to be mixed well. 	



Appendix B Warranty

Digital Bio warrants that the ADAM will be free from defects in material and workmanship for a period of one (1) year from date of purchase.

If any defects occur in the ADAM during this warranty period, Digital Bio will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

- 1. Defects caused by improper operation.
- 2. Repair or modification done by anyone other than Digital Bio or an authorized agent.
- 3. Damage caused by substituting alternative parts.
- 4. Use of fittings or spare parts supplied by anyone other than Digital Bio.
- 5. Damage caused by accident or misuse.
- 6. Damage caused by disaster.
- 7. Corrosion caused by improper solvent or sample.

For your protection, items being returned must be insured against possible damage or loss. Digital Bio cannot be responsible for damage incurred during shipment of a repair instrument; It is recommend that you save the original packing material in which the instrument was shipped. This warranty should be limited to the replacement of defective products.

For any inquiry or request for repair service, contact sales@digital-bio.com or your local distributor.



Appendix C Technical Specifications











<u>ADAM</u>

Voltage: AC100~240 V, 50~60 Hz Current: max. 1.8 A, max 100 W Fuse: F3.15AL250V Objective lens: 4 X LED: 4W Green LED IEC 60825-1: 1993+A1;1997+A2;2001 CCD camera: B/W CCD Filter: Excitation filter, Dichroic filter, Emission filter Weight: 9 Kg Size (W×L×H): 220 × 375 × 250 mm Degree of protection : IPX0

AccuChip

Measuring range: 0 to 1×10^7 cells/mL Analysis time: 2 ~ 2.5 min/test Loading sample vol.: 20 µL (for AccuChip 2^x) 12 µL (for AccuChip 4^x)/test Measuring vol.: 8.5 µL (for AccuChip 2^x) 3 µL (for AccuChip 4^x)/test

Somatic cell counting SCC Kit

(CRS-K01)

Accessories

Power cord: 1.5 m Fuse: 250 VAC, 3 A; F3.15AL250V Keypad External video monitor (optional)

Environment Condition

 $5 \le T \le 30$ °C Altitude ≤ 2000 m

Contact Information

E-Mail : sales@digital-bio.com Internet: www.digital-bio.com

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