BioFocus® Capillary Electrophoresis System Verification Kit

Instruction Manual

Catalog Number 148-4170

BIO-RAD

For Technical Service Call Your Local Bio-Rad Office
or in the U.S. Call **1-800-4BIORAD** (1-800-424-6723)

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Section 1
Introduction

The BioFocus capillary electrophoresis system verification kit is used to verify the performance of the BioFocus capillary electrophoresis system. It consists of a verification test sample, run buffer, and two lengths of fused silica capillary. When used in conjunction with BioFocus software version 5.2 or higher, the verification process is completely automated, producing a verification report that indicates a pass/fail with statistical data on the performance of the system. Earlier versions of the software require that the configuration, methods, and automation sequence be programmed manually.

When analyzed using the conditions described in this manual, the test sample produces a single sharp peak. It has been selected for its stability and minimal interactions with the capillary under the test conditions, and so provides a convenient and reliable method for evaluating the performance of the instrument without variations in the separation chemistry. Consistent behavior of the test compound in replicate injections therefore confirms proper adjustment and operation of all instrument parameters contributing to satisfactory analytical precision such as injection pneumatics, capillary temperature control, high voltage power, and detector performance.

The kit is intended for use with the User Assembled Capillary Cartridge (catalog number 148-3052). It is assumed that the oper-

ator is familiar with the use of this cartridge and the operation of the BioFocus capillary electrophoresis system. If not, refer to the instruction manuals for these products.

Section 2
Kit Components

Verification Test Sample - 30 ml of 1 mg/ml benzene sulfonic acid in dilute acidic phosphate buffer

Run Buffer - 2 x 60 ml of 100 mM sodium phosphate, pH 2.5 with hydrophilic polymer

Capillary - BioCAP™ fused silica capillary, 50 µm ID x 360 µm OD x 40 cm

Section 3
Reagent Preparation

The run buffer and test sample should be brought to room temperature before use. All reagents should be put into 500 µl microcentrifuge tubes in the quantities shown below.

Qty	Reagent	Volume	ID
3	Run Buffer	500 µl	PO4_RUN (2),PO4_PREP
2	Water	500 µl	Water (Inlet and Outlet)
1	Water	150 µl	Waste
1	Test Sample	150 µl	Verification Test Sample

These vials should be placed into the carousels as indicated in the configuration shown in Table 1.

Section 4
Capillary Preparation

Install the capillary into the User Assembled Cartridge using a length of polyurethane cooling tubing cut to 10.1 cm to provide a total capillary length of 24 cm. Insert the cartridge into the instrument.

Section 5
Performing the Verification Test

Using BioFocus software version 5.2 or higher, system verification can be completely automated by executing a preprogrammed automation sequence labeled VERIFY__. This automation sequence links three separate methods. The first method (EQUIL) performs a blank 12-minute run with no injection to insure that the instrument is fully warmed up and equilibrated. The second method (VERIFY) is used to sequentially inject the test

sample for a total of 10 replicate 6-minute runs. The third method (FINAL) automatically purges buffer from the capillary, flushes it with water and nitrogen, turns off the detector lamp and leaves the instrument in an idle state.

When using earlier versions of the BioFocus operating software, the cartridge, configuration, methods, and automation sequence listed in Tables 1–5 should be manually entered into the database.

When installing version 5.2 software, the pre-existing database (configurations, methods, etc.) can be preserved if you wish to continue working with previous methods; in this case the information in Tables 1–5 should be entered manually after installation of the new operating software.

The instrument should be turned on for 1 hour prior to initiating the test. The detector lamp is turned on automatically at instrument startup; if the instrument is already on but its prior history is not known, confirm that the lamp is on and wait for 1 hour before starting the test. For systems equipped with carousel compartment temperature control, set this value to 23 °C.

Deselect integration between runs (make sure the automatic integration icon in the main screen toolbar is in the deselected mode). Start the automation sequence and allow approximately 1.5 hr for completion. The BioFocus software stores the verification data files in a subdirectory named VERIFY in the root directory of the the C drive.

Section 6
Data Reduction

Calculation of migration time and peak area precision can be performed automatically using BioFocus integration software version 5.2 or higher. Open the integrator program and click on the "Verification" button at the top right side of the File Open window. The software will automatically open the most recent ten VER__ data files, analyze the data, and generate a report containing the migration times and peak areas for the test compound in the ten replicate runs. The relative standard deviations for migration times and peak areas will be calculated and compared to the Performance Standard values listed in Section 7; a typical Verification Report is presented in Table 6.

If you are using a version of the BioFocus integrator software earlier than 5.2, the verification report must be generated manually. To accomplish this, open the program when the automation sequence is completed. Program an integration method by editing the Default method and saving it under a new name (*e.g.* "VERIFY"), or by creating a new method. In this method, change the value of Threshold from 0.02 to 0.15 on the Integration Parameters page, and change the final time to 6.00 min on the Timed Events page. Deselect "Print Electropherogram" on the Electropherogram page, if desired, to save time or printer paper. Return to the File Open window; locate the ten datafiles (ver001.bff, ver002.bff, etc.) in the file listbox and enter into the integration listbox. Select "Extract

Run Histories" if desired (this provides useful troubleshooting information if the resulting data are unsatisfactory). Select the VERIFY integration method for each file and analyze the data set. When analysis is completed, open the Report1.txt file (this can be done easily using the View Reports feature in the Electropherogram Database window File menu) and copy peak areas and migration times for the ten runs into an appropriate statistical program or spreadsheet for calculation of mean, standard deviation and % relative standard deviation (RSD).

Section 7
Performance Standard

A BioFocus system and capillary cartridge in satisfactory operating condition should exhibit migration time precision of 1.5 %RSD or better and peak area precision of 2.5 %RSD or better when the system verification is performed using the BioFocus system verification kit exactly according to the above instructions.

Section 8
Product Information

Catalog Number	Product Description
148-4170	**BioFocus System Verification Kit**
148-5010	**Phosphate Buffer,** 4 x 60 ml
148-3060	**BioCAP Fused Silica Capillary,** 2 x 40 cm x 50 µm ID, uncoated, window at 8 cm
148-3052	**BioFocus User Assembled Cartridge**

Table 1. Verification Configuration

ID: VERIFY Description: System Verification

INLET CAROUSEL POSITIONS			OUTLET CAROUSEL POSITIONS		
Pos	**Type**	**ID/Description**	**Pos**	**Type**	**ID/Description**
1	R	PO4_PREP	1	W	Waste
2	R	PO4_RUN	2	R	PO4_RUN
3	S	Verification Test Sample	3	R	WATER
4	R	WATER	4		
5	R	NITROGEN	5		

CARTRIDGE DATA

Catalog Number: UAC Serial Number: VERIFY

Length: 24 cm Diameter: 50 µm Uncoated

Use Count: 0 Active

Table 2. Equilibration Method Parameters

ID: EQUILIBRATE Description: Equilibration Method
Prep 1: Pre-Inject from [PO4_PREP] to [Waste] 45 sec
No Injection
Polarity: Negative - >Positive
Run Voltage: 10.00 kV
Current Limit: 100.00 µA
Inlet buffer: [PO4_RUN .....]
Outlet buffer: [PO4_RUN .....]
Cartridge Temperature: 30 degrees Celsius
Run Time: 12.00 min
Single Wavelength Programmable Detector Mode

Start (min)	End (min)	Lambda (nm)	Ch'nl	Range	R-Time (sec)
0.00	12.00	200	1	0.0200	1.0

Zero upon wavelength change
Scheduled Zeros (min): None
R-Time (sec): 1.0
Lamp(s) remain on at the end of the run group

Table 3. Verification Method Parameters

ID: VERIFY Description: Verification Method
Prep 1: Pre-Inject from [PO4_PREP] to [Waste] 30 sec
Low Pressure Injection: 8 psi * sec
Polarity: Negative - >Positive
Run Voltage: 8.00 kV
Current Limit: 75.00 µA
Inlet buffer: [PO4_RUN.....]
Outlet buffer: [PO4_RUN.....]
Cartridge Temperature: 22 degrees Celsius
Run Time: 6.00 min
Single Wavelength Programmable Detector Mode

Start (min)	End (min)	Lambda (nm)	Ch'nl	Range	R-Time (sec)
0.00	6.00	200	1	0.0200	1.0

Zero upon wavelength change
Scheduled Zeros (min): None
R-Time (sec): 1.0
Lamp(s) remain on at the end of the run group

Table 4. Final Method Parameters

ID: FINAL Description: Terminates Autosequence

Prep 1: Pre-Inject from [WATER] to [Waste] 60 sec

Prep 2: Pre-Inject from [NITROGEN] to [Waste] 300 sec

No Injection

Polarity: Negative - >Positive

Run Voltage: 0.00 kV

Current Limit: 0.30 µA

Inlet buffer: [WATER .....]

Outlet buffer: [WATER .....]

Cartridge Temperature: 20 degrees Celsius

Run Time: 1.00 min

Single Wavelength Programmable Detector Mode

Start (min)	End (min)	Lambda (nm)	Ch'nl	Range	R-Time (sec)
0.00	1.00	200	1	0.0200	1.0

Zero upon wavelength change

Scheduled Zeros (min): None

R-Time (sec): 1.0

Lamp(s) turned off at the end of the run group

Table 5. Verification Automation Sequence

ID: VERIFY__ Configuration ID: VERIFY

Number of Run Groups: 3 Number of Runs: 12

Sample Range Group	Run Sets	Method ID	Run Group Status	Waste Vial	Run Description
3/3	1	EQUIL	Ready	1	Syste Verification
3/3	10	VERIFY	Ready	1	Syste Verification
3/3	1	FINAL	Ready	1	Syste Verification

Table 6. Verification Report

BioFocus System VERIFICATION REPORT

Report Date	Fri Mar 01 16:14:03 1996
Instrument Model	BioFocus 3000TC
Instrument Serial #	951BR0038
Instrument Hardware ID	0101
Z180 Firmware Rev.	J
HC11 Firmware Rev.	F
Operating Software	Unreleased: X-VAL-002
Operator Name	Tim Wehr
Institution	CE Applications Laboratory Bio-Rad Laboratories 2000 Alfred Nobel Drive, Hercules, CA

Comments

OPERATING PARAMETERS

Capillary	24 cm x 50 um coated
Capillary Temp	22 deg C
Sample	verification test sample
Inlet	148-5010 phosphate, 0.1M
Outlet	148-5010 phosphate, 0.1M
Injection	8 psi*sec
Run Voltage	8.00 kV - to +
Detector	Single wavelength at 200 nm
Carousel Temp	20 deg C

TEST DATA

File Name	Migration Time	Peak Area	Date/Time
VER__044	3.17	358075	Fri Mar 01 12:15:54 1996
VER__045	3.18	346201	Fri Mar 01 12:24:10 1996
VER__046	3.18	349941	Fri Mar 01 12:32:26 1996
VER__047	3.18	345425	Fri Mar 01 12:40:40 1996
VER__048	3.19	359233	Fri Mar 01 12:48:56 1996
VER__049	3.18	360125	Fri Mar 01 12:57:10 1996
VER__050	3.18	352467	Fri Mar 01 13:05:24 1996
VER__051	3.19	354192	Fri Mar 01 13:13:38 1996
VER__052	3.18	343057	Fri Mar 01 13:21:54 1996
VER__053	3.17	351593	Fri Mar 01 13:30:08 1996
MEAN	3.178	352030	
STD DEV	0.008	5976	
RELATIVE STD DEV	0.24%	1.70%	
ACCEPTABLE RSD	1.50%	2.50%	
RESULTS	PASS	PASS	

Signature:_____ Date:_____

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