EQUIPMENT SPECIFICATION

Page 1 of 4

Title: GRADIFRAC SYSTEM		Document Number: ES1704		
Supersedes/Date: 2000-11-20	CO No.: 10-0454	Revision: 02		
QA Approval/Date: Dan Simpson 2010-08-04		Effective Date: 2010-08-06		

1. INTRODUCTION

This document describes the proper use and maintenance of the GradiFrac System (EQ#064) and its associated columns with flow adaptor (EQ#065). The GradiFrac System from Pharmacia Biotech AB combines the functions of gradient programmer and programmable fraction collection for standard chromatography applications.

The GradiFrac System is composed of several components. The GradiFrac instrument can be programmed to collect fractions based on time, volume, drop count or in a peak fraction mode when the monitor is in use. The Pump P-1 is a single channel laboratory pump for use in liquid chromatography for accurately controlled liquid flows. The Monitor UV-1 is a fixed wavelength monitor to measure the absorbance of a flowing liquid. The Recorder REC 102 allows charting during fraction collections. The Valve IV-7, Switch Valve PSV-50, and Chromatography Column with Flow Adaptor are also components.

This document does not address preparing, regenerating, or storing columns. This information is covered in the appropriate manufacturing documents.

2. **RESPONSIBILITY**

The user shall be responsible for the proper use of the GradiFrac System. The department in which the instrument resides shall be responsible for its maintenance.

3. REFERENCES

GradiFrac System Installation Guide, Pharmacia Biotech publication number 18-1035-18, Edition AD, 1994

GradiFrac User Manual, Pharmacia Biotech publication number 56-1006-08, Edition AC, 1994 Monitor UV-1 User Manual, Pharmacia Biotech publication number 59-7797-01, Edition AF, 1994

GradiFrac System with HiLoad Pump P-50 Installation Guide, Pharmacia Biotech publication number 18-

1103-53, Edition AA, 1994

Peristaltic Pump P-1 User Manual, Pharmacia Biotech publication number 59-4698-01, Edition AG, 1995

- Recorder REC 101/102 User Manual, Pharmacia Biotech publication number 80-1300-59, Edition AE, 1994
- Columns C10, C16, C26 Instructions, Pharmacia Biotech publication number 59-5261-00, Edition AH

Adaptors AC 10, AC 16, AC 26 Instructions, Pharmacia Biotech publication number 56-1021-49, Edition AD

Solenoid Valve PSV-50 Instructions, Pharmacia Biotech publication number 19-1994-05, Edition AD, 1995

Valve IV-7 Instructions, Pharmacia Biotech publication number 19-1996-05, Edition AC, 1993

4. SAFETY

Always disconnect all instruments from their power source before performing any maintenance.

Page 2 of 4

Title: **GRADIFRAC SYSTEM**

Document Number: ES1704

Revision: 02

Effective Date: 2010-08-06

5. EQUIPMENT/MATERIALS

Kimax 51 borosilicate disposable glass culture tubes, 18 X 150 mm, VWR #60825-673 or equivalent

6. **PROCEDURE**

Note: To use the GradiFrac system, you must have a packed column. Refer to the appropriate manufacturing document.

- 6.1 Operation
 - 6.1.1 Monitor UV-1
 - 6.1.1.1 Turn the Control Unit on.
 - 6.1.1.2 Select the desired Absorbance range as specified in the appropriate manufacturing document.
 - 6.1.1.3 Make sure that the mode switch is in the AU mode.
 - 6.1.2 Connecting the Chromatography Column
 - 6.1.2.1 Connect the chromatography column between port 1 of the IV-7 valve and the lower left port of the UV-1 cell flow.
 - 6.1.2.2 Place the "A" tubing coming from the PSV-50 valve in the solution to be run through the column.
 - 6.1.3 Recorder
 - 6.1.3.1 Install a colored pen by gently pushing it into the lower metal pen holder.
 - 6.1.3.2 Select the desired chart speed with the chart speed switch as specified in the appropriate manufacturing document.
 - 6.1.3.3 Make sure that the "Rec off/on" key is in the down position. The recorder will then start when the flow is started and stop when the flow is stopped.
 - 6.1.4 Pump P-1

Make sure that the pump is on. The pump will start when the flow is started and stop when the flow is stopped.

Title: GRADIFRAC SYSTEM

Document Number: ES1704

Revision: 02

Page 3 of 4

Effective Date: 2010-08-06

- 6.1.5 Fraction Collector
 - 6.1.5.1 Move the delivery arm up and out.
 - 6.1.5.2 Insert the collection tubes in the tube rack.
 - 6.1.5.3 Lift the delivery arm a little and move it so that the tube sensor rests against a tube.
- 6.1.6 Running the system
 - 6.1.6.1 The display of the GradiFrac System should show "Main Menu RUN METHOD". Press the down arrow until "Main Menu MANUAL RUN" is showing. Press ENTER.
 - 6.1.6.2 Press the down arrow until "Manual Flow mL/min" is displayed. Enter the desired flow rate as specified in the appropriate manufacturing document (e.g., 5.0). Press ENTER. The pump will now start drawing the liquid.
 - 6.1.6.3 The display now shows "MANUAL FRACTION mL". Press ENTER and enter the desired volume (e.g., 2.0) when it is time to start collecting. Everything should now be working. If drops of liquid do not begin to flow into the first collection tube, press "END". The display will read "Main Menu RUN METHOD". Repeat section 6.1 until desired material has been eluted.
- 6.1.7 Documentation

The user shall complete the GradiFrac System Log (**ES1704-1**). If a new log sheet is required, the user shall record the number of hours run since the last tubing replacement. If the Pump P-1 tubing has just been replaced, the number of hours run shall be recorded as zero. Forward completed log sheets to QC for review. File log sheets in the EQ file (EQ# 064).

6.2 Maintenance

- 6.2.1 The GradiFrac should be cleaned thoroughly after each use. The surface may be wiped with aqueous cleaning solutions or with ethanol. The delivery arm should always be returned to the central position over the bowl when not in use. Document the cleaning on **ES1704-1**.
- 6.2.2 Tubing on the peristaltic pump P-1 should be replaced after 200 hours to minimize the risk of tube breakage during operations. Document tubing replacement on **ES1704-1**.
- 6.2.3 To ensure trouble free operation of the monitor, the following precautions should be taken:
 - 6.2.3.1 All liquids through the flow cell should be free of suspended particles.
 - 6.2.3.2 Never allow buffer solutions to dry out in the flow cell.

EQUIPMENT SPECIFICATION

Title: **GRADIFRAC SYSTEM**

Document Number: ES1704

Revision: 02

Effective Date: 2010-08-06

- 6.2.3.3 All liquids should be degassed to prevent air bubble formation in the flow cell.
- 6.2.3.4 Always rinse the flow cell thoroughly with distilled water after use.
- 6.2.3.5 Never touch the optical surfaces of the interference filters or expose them to temperatures greater than or equal to 60°C.
- 6.2.4 The recorder requires minimum maintenance. No lubrication is necessary. Never lubricate the guide axis of the pen carriage. Clean the guide axis periodically with a soft cloth to remove dust. When changing the chart paper, remove any excessive dust.
- 6.2.5 When finished with a run, reconnect tubing between port 1 of the IV-7 valve and the UV-1 flow cell. This creates a closed system to prevent dust in the GradiFrac.
- 6.3 Troubleshooting

Reference the operation manuals for each specific component part for troubleshooting suggestions.

7. ATTACHMENTS/APPENDICES

GradiFrac System Log (ES1704-1)

REVISION HISTORY						
Revision	Description	CO	Effective Date			
02	Removed O-ring replacement Removed run rate and fraction columns from log	10-0454	2010-08-06			

GRADIFRAC SYSTEM LOG

Number of hours run since last tubing replacement:

Date	User	Run Time	Use	Cleaned ($$)

Peristaltic Pump P-1 Tubing

 Total number of hours run*:
 Date:
 Technician:

 *If the total number of hours run is \geq 200, replace the tubing.
 Technician:

Tubing Replaced: Yes No

QC Review: _____

Date: _____