Agilent Technologies

G1968C Mass-Based Fraction Collection Kit Installation Instructions

Follow the steps below to install the G1968C Mass-Based Fraction Collection (MBFC) kit. This kit is designed to be used as part of an Agilent 1100 Series Purification/High Throughput system when an LC/MSD is used as a detector to generate a trigger signal for fraction collection. This kit can be used with either a preparative scale (<100 ml/min LC flow) or analytical scale (<10 ml/min LC flow) 1100 LC system, and can be used with a G1946B, G1946C or G1946D LC/MSD.

The G1968C mass-based fraction collection kit includes the following items:

G1946-60166	Internal I/O cable
G1946-65016	I/O Accessory PCA
G1968-60002	LC/MS Fraction Collection Control Cable (from I/O connector to UIB)
0100-1516	Fingertight fitting
G1968-60500	Tubing, 1/16" o.d. x .040" i.d., 1524 cm
0890-1762	PEEK tubing, 1/16" o.d. x .010" (0.25mm) i.d.
0100-0900	Union, zero dead volume, 1/16"
G1390-64000	Universal Interface Box (to order separately, order product number G1390A)
G1364-63104	Internal tray assembly with flow delay sensor, for G1364A preparative scale fraction collector
G1968-64102	Rheodyne MRA™ active splitter kit (to order separately, order standalone active splitter p/n G1968-64104)
59987-20033	Electrospray LC Demo Sample
G1946-85020	Delay Calibrant
846975-202	Zorbax SB-C18 column, 5 um, 9.4 x 50 mm
G2263AA	Mass-based fraction collection add-on software (license packet only)
G2264AA	Purity Check/HiThruput add-on software (license packet only)

Install I/O Accessory PCA and Internal I/O cable

If the G1968C mass-based fraction collection kit is to be used with a G1946C or G1946D LC/MSD, the I/O accessory PCA must first be installed in the LC/MSD. If the mass-based fraction collection kit is to be used with a G1946B LC/MSD, then you can skip the following steps, since the G1946B LC/MSD already includes the I/O accessory PCA.

1. Close the LC/MSD ChemStation session.

2. Remove the front, top, and left side covers.

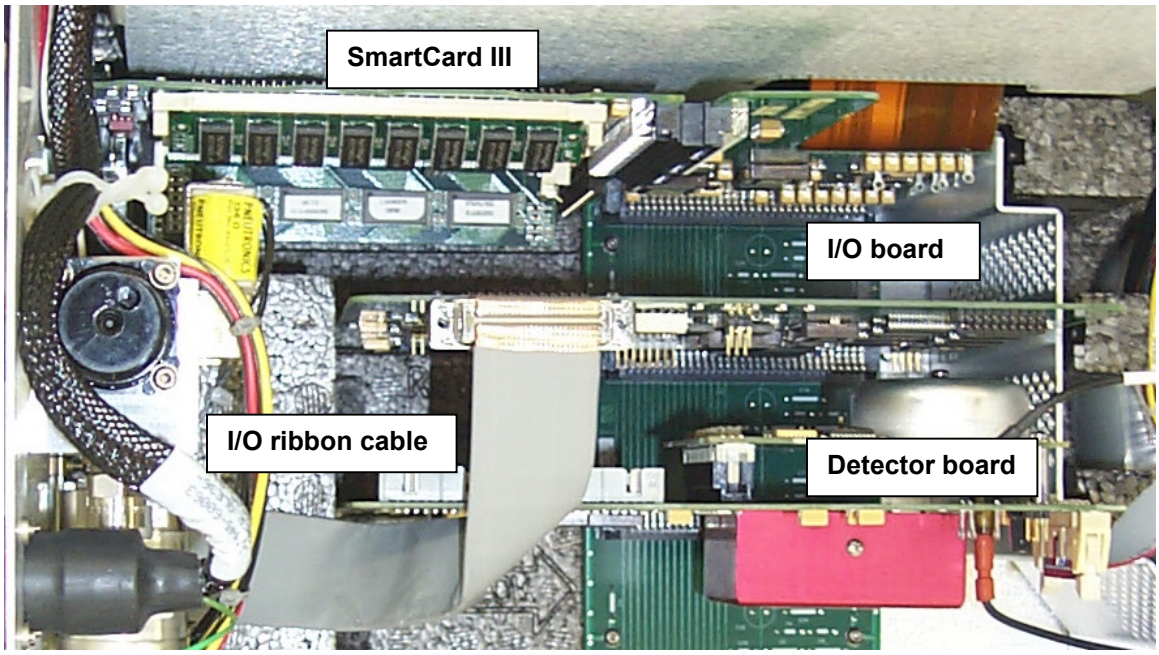
3. Remove the top electronics cover and the top foam piece.

4. Remove the RF coil box, RFPA assembly, and the middle foam piece.

5. Use a 3/16" or 5 mm nut driver to remove the I/O cover plate from the side panel. Save the standoffs for use when installing the internal I/O cable.

6. From the inside of the electronics tub, install the I/O ribbon cable supplied in the kit into the slot in the side panel. Use the two standoffs that you removed in the previous step.

7. Install the I/O board supplied in the upgrade kit into the connection in the backplane next to the SmartCard III.



8. Connect the I/O ribbon cable to the connector at the top of the I/O board. The ribbon cable should fold over the detector board.

9. Reinstall the middle foam piece and make the additional connections from the analyzer and detector boards to the other components.

10. Reinstall the RFPA and RF coil box.

11. Reinstall the top foam piece and the electronics cover.

12. Reinstall the front, top, and side covers.

Install Universal Interface Box

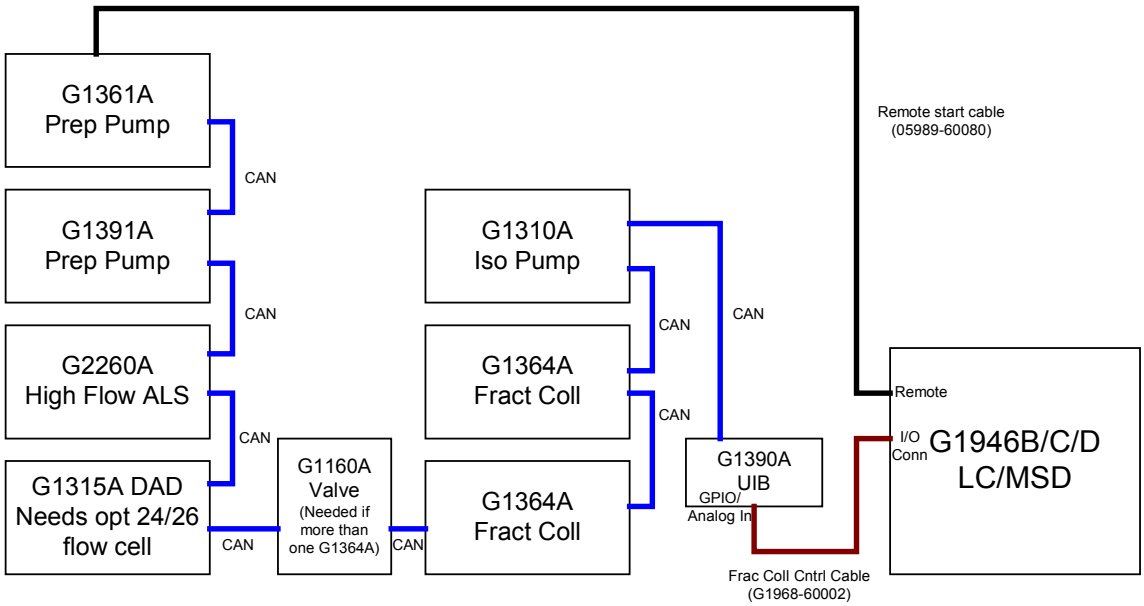
1. Place the Universal Interface Box (UIB) from the G1968C kit on the lab bench to the left of the LC/MSD.

2. Connect the fraction collection cable (p/n G1968-60002) from the 15-pin GPIO/Analog In connector on the front of the UIB to the 25-pin I/O accessory connector on the left-side panel of the LC/MSD.

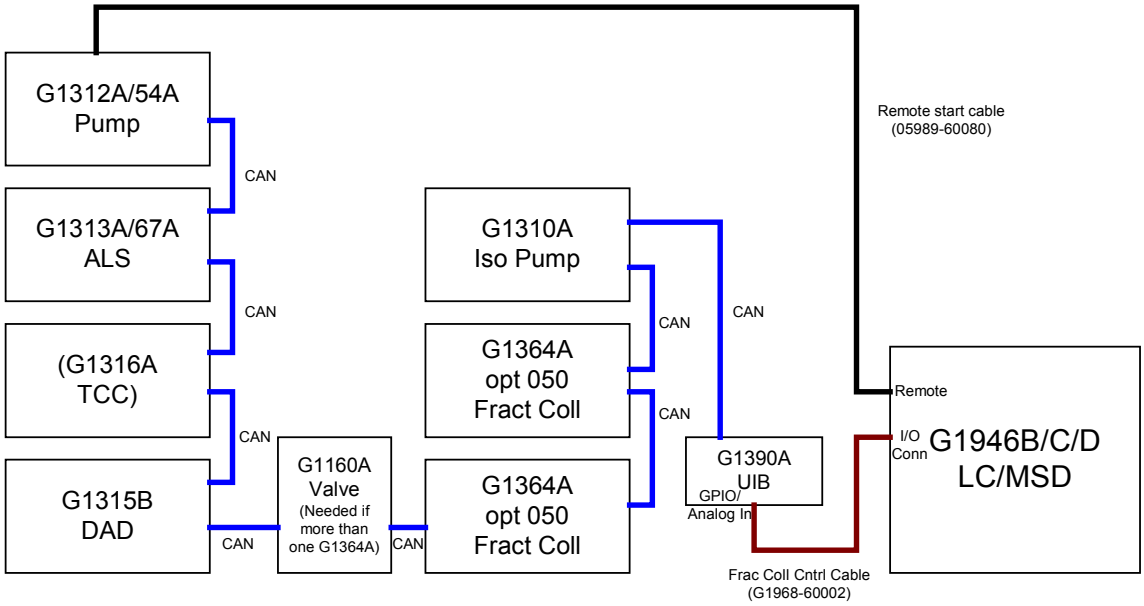
3. Connect the CAN cable supplied with the UIB from one of the CAN connections on the rear of the UIB to an open CAN connection on one of the 1100 LC modules.

4. If the UIB will be used to process the analog signal from an LC detector, then connect the analog signal cable from the LC detector to the Analog In BNC connection on the front of the UIB.

Cabling—Prep Scale System



Cabling—Analytical Scale System

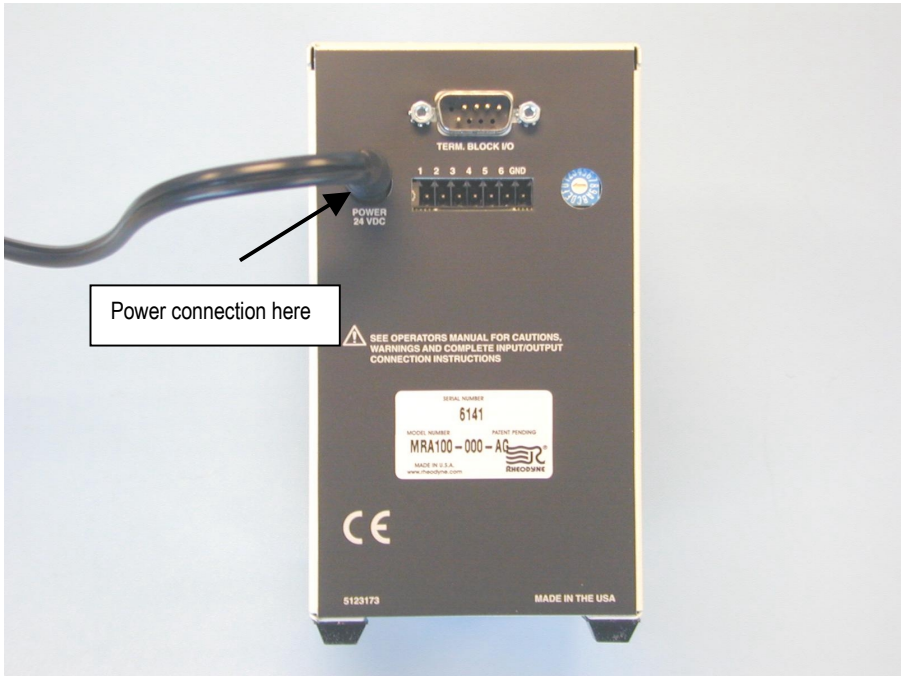


Set up Active Splitter

1. Unpack the Rheodyne MRA™ (Mass Rate Attenuation) active splitter and place it on the lab bench in a location between the 1100 LC detector and the LC/MSD.

Electrical Connections

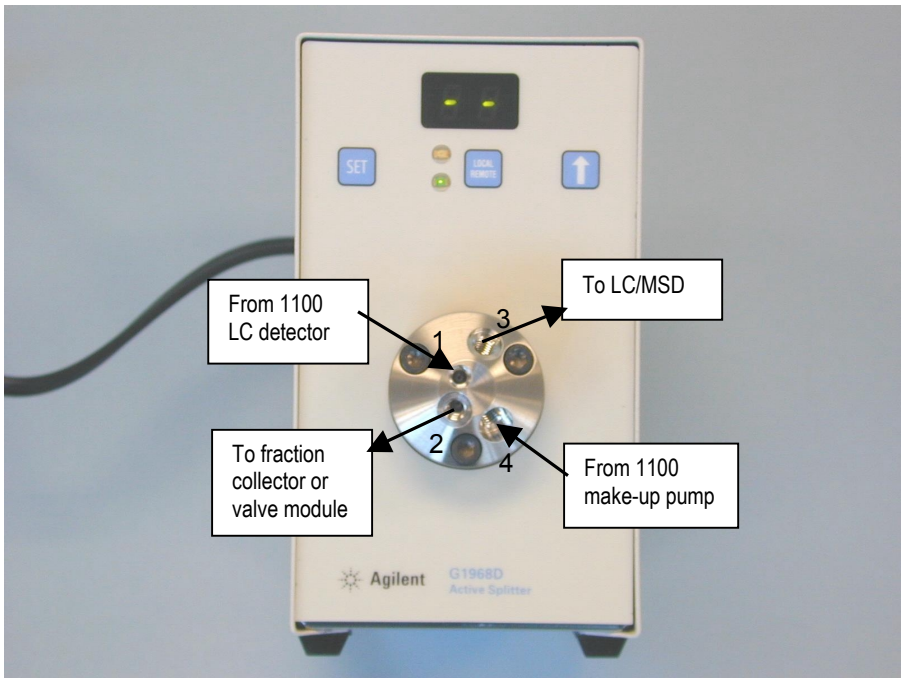
2. Connect the output of Universal Power Supply shipped with the splitter to the Power/24 VDC connection on the back of the splitter.



3. Plug the power cord supplied in the mass-based fraction collection kit into the universal power supply, and then plug the power cord into the wall outlet (Note: no power cord is supplied with the G1968-64102 Rheodyne MRA™ active splitter kit; use the one supplied in the mass-based fraction collection kit). The universal power supply can be operated from inputs of 100-240 Vac, 50-60 Hz. The output is 24 Vdc, 1.7 A.

Plumbing Connections

The plumbing connections to the splitter are made on the front to ports 1-4 of the valve. Four fingertight fittings are supplied with the splitter and should used for connecting the tubing



1. Connect the tubing from the 1100 LC detector to port 1 on the front of the splitter. This tubing should be the tubing supplied with the 1100 LC modules and of the appropriate size to accommodate the LC flow.

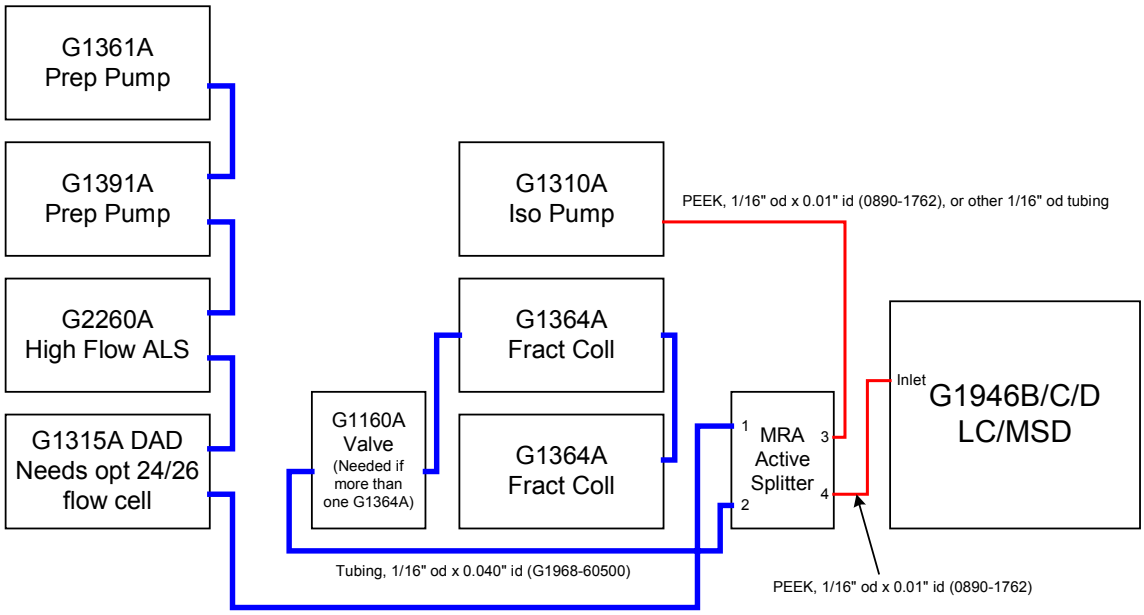
2. Cut a length of the 1/16" PTFE tubing (p/n G1968-60500) supplied in the kit and connect it from port 2 on the front of the splitter to either the G1364A fraction collector module or, if more than one fraction collector module is configured, connect the tubing to the G1160A Valve module.

   Note: the length of the tubing will determine the delay time for a compound to reach the fraction collector. It is important that this time be longer than the time required for the compound to reach the MSD. The length of tubing used will depend on the LC flow rate. As an approximation, if the LC flow rate is 5 ml/min, then 2 m of tubing will be required; if the LC flow rate is 100 ml/min, then the full 15 m of tubing should be used.

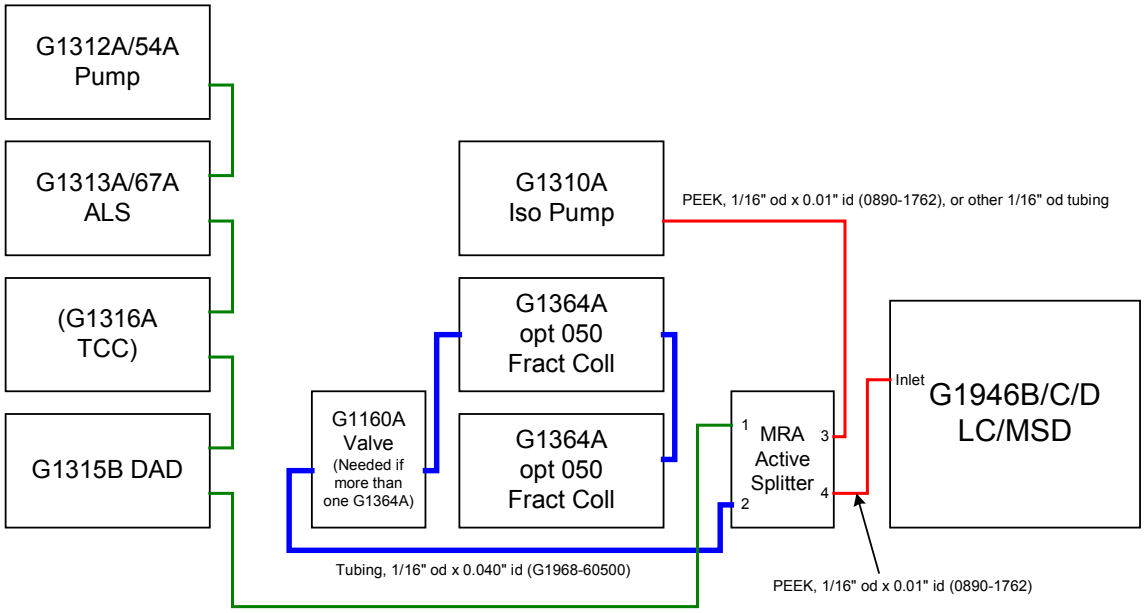
3. Connect a length of PEEK tubing (p/n 0890-1762) from port 3 to the inlet fitting on the LC/MSD. Try to use as short a length of the tubing as possible from the splitter to the LC/MSD, in order to minimize the delay time associated with the MS detector. For minimum delay, the splitter can be placed directly on top of the MSD and a short piece of tubing plumbed directly between the splitter and the nebulizer of the MSD. For tuning, remember to reconnect the original tubing to the nebulizer.

4. Connect a separate length of PEEK tubing (p/n 0890-1762) or other 1/16" od tubing from port 4 to the make-up pump used for the splitter. The make-up flow will ensure sufficient flow from the splitter to the LC/MSD.

Plumbing Diagram—Prep Scale System



Plumbing Diagram—Analytical Scale System



Setting Split Flow

1. Make sure the splitter is in local mode (yellow LED = local; green LED = remote). If the splitter is in the remote mode, hit the LOCAL/REMOTE button to change it to local mode.

2. Press the SET button.

3. The table below provides a list of split factors corresponding to a particular LC flow rate and desired split ratio. Use the Forward Position Selector button (the up arrow) to set the split factor you want.

Split Factors

	Split Ratio						
LC Flow Rate (ml/min)	**100:1**	**500:1**	**1,000:1**	**4,000:1**	**10,000:1**	**20,000:1**	**100,000:1**
1	1	4	12				
2	2	5	13	22			
4	3	6	14	23	34		
6		7	15	24	35	45	
8		8	16	25	36	46	
10		9	17	26	37	47	
15		10	18	27	38	48	
20		11	19	28	39	49	
30			20	29	40	50	55
40			21	30	41	51	56
60				31	42	52	57
80				32	43	53	58
100				33	44	54	59

4. Press the SET button again until -- is displayed. The split factor is now set in the splitter.

Note: For LC flow rates not listed in the table, refer to the Rheodyne "MRA™ Operating Manual" for information on calculating the corresponding split ratio for a given split factor.

Operating the Active Splitter Manually

To manually turn on the active splitter (split flow going to LC/MSD), press both the SET button and the Forward Position Selector button (the up arrow) at the same time.

To turn the splitter off (no split flow going to LC/MSD, all flow going to fraction collectors/valve), press both the SET button and the Forward Position Selector button (the up arrow) at the same time again.

Operating the Active Splitter Automatically

The active splitter can be turned on and off automatically through a contact closure on the back Terminal Block connector of the splitter. A G1351A External Contacts Board can used to provide the contact closure.

1. Install the G1351A External Contacts board (not provided in the mass-based fraction collection kit; must be purchased separately) in an available slot in one of the 1100 LC modules.

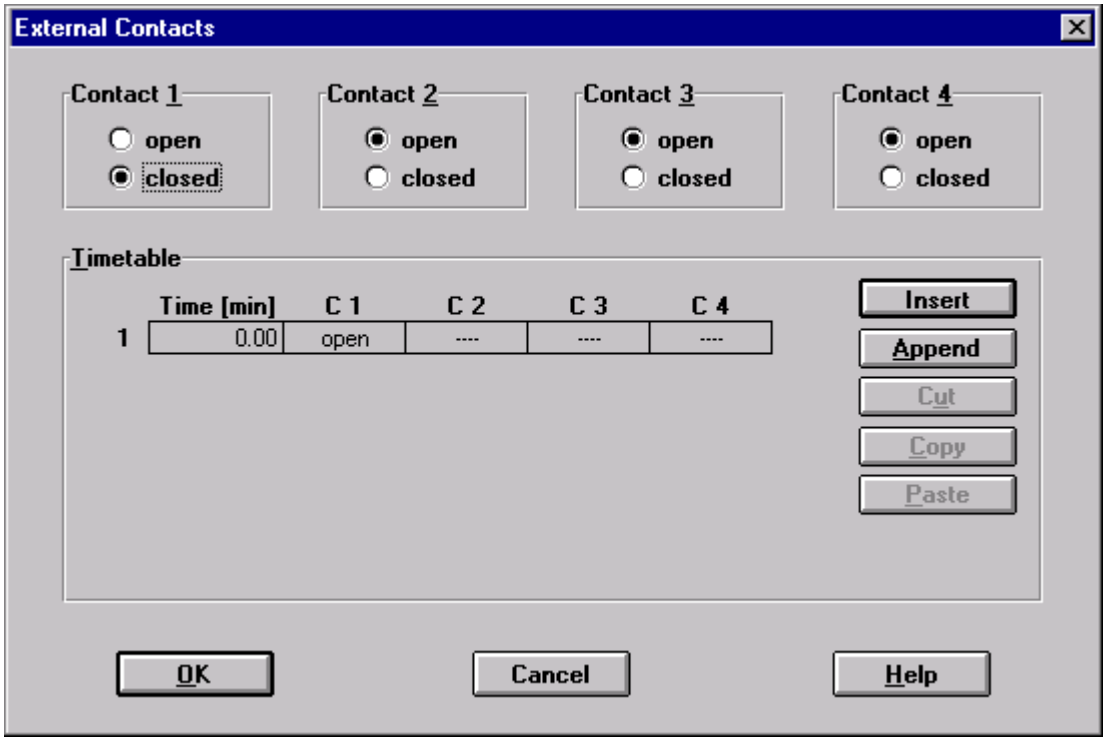
2. Connect the G1103-61611 general purpose cable to the connector on the external contacts board

3. Connect the brown wire on the other end of the general purpose cable to terminal 7 (GND) of the Terminal Block connector on the back of the active splitter.

4. Connect the white wire of the general purpose cable to terminal 4 of the Terminal Block connector on the back of the active splitter.

The operation of the active splitter can be now controlled through the external contacts dialog box in the ChemStation method. To set external contacts, first make sure Full menu is selected, then select Instrument, then More Gradient Prep Pumps... (or whichever 1100 LC module the External Contacts Board is installed in), then Contacts B.... Use an initial statue of closed for contact 1, and

then open at Time=0.00. The active splitter will then start at the beginning and stop at the end of each run.



Installing the Internal Tray Assembly in the Preparative Scale Fraction Collector

If the system is configured with a preparative scale fraction collector, install the G1364-63104 internal tray assembly supplied in the mass-based fraction collection kit into the preparative scale fraction collector. Refer to the "Agilent 1100 Series Fraction Collectors Reference Manual", p/n G1364-90000, for information on installing the internal tray assembly into the fraction collector.

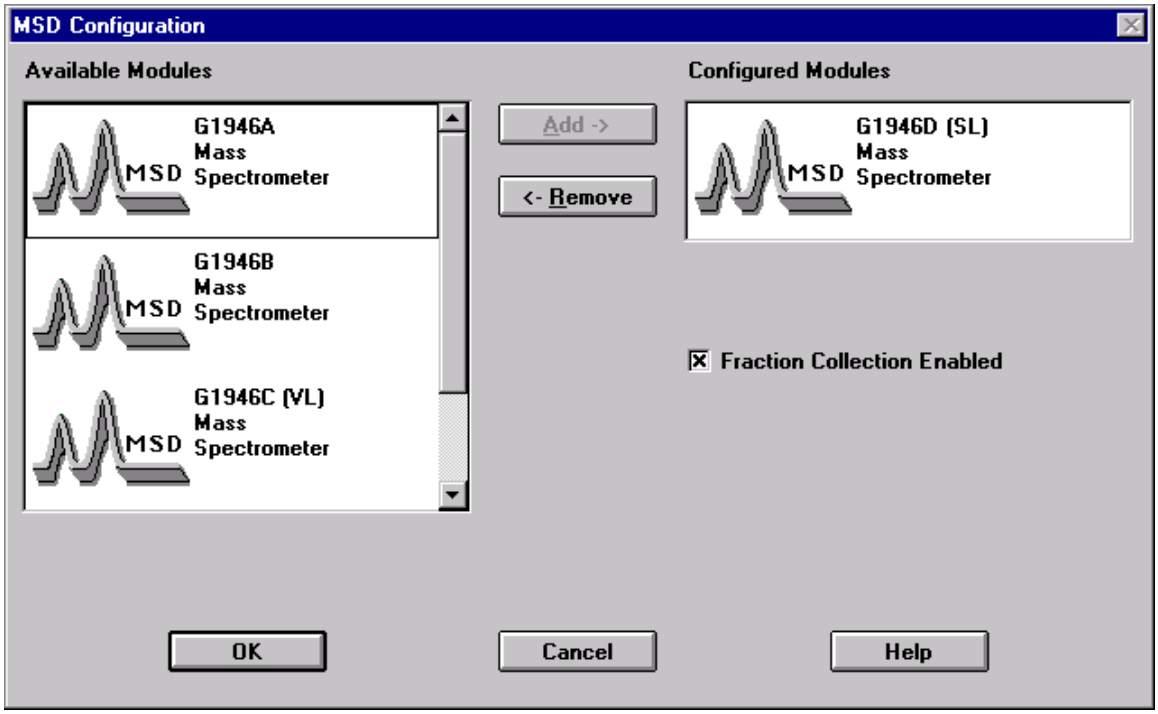
If the system is configured with an analytical scale fraction collector, you do not need to install the tray, since the analytical scale fraction collection comes with the tray already installed.

Installing the G2263AA/G2264AA software

The mass-based fraction collection kit includes software license packets for the G2263AA mass-based fraction collection add-on software and the G2264AA Purity Check/HiThruput add-on software. These products should be installed when the G2262AA Purification/HiThruput software is installed. Follow the instructions given in the "User's Guide for Purification/High Throughput Systems" (p/n G2262-90001) to install the G2262AA/63AA/64AA software.

Configuring the LC/MSD ChemStation for mass-based fraction collection

To configure the LC/MSD ChemStation software for mass-based fraction collection, select Instrument/Configure 1100 MSD..., then make sure the Fraction Collection Enabled box is checked.



Delay sensor calibration

Follow the instructions given in the "User's Guide for Purification/High Throughput Systems" (p/n G2262-90001) to calibrate the delay time for either an analytical scale or a preparative scale system.

The delay time should be determined at each LC flow rate. The delay time is not dependent on solvent composition.

When determining the delay time, the LC column should be removed from the flow path.

To determine the delay time:

1. Create or modify a flow sensor method so the LC flow setting is consistent with the desired MSD method conditions. The split factor set on the active splitter should also correspond to the split ratio used in the MSD method.

2. Run the delay sensor calibration and observe the time for the MSD TIC peak relative to the delay sensor.

3. If the delay sensor peak elutes AFTER the MSD peak, take the time difference and put it into the collector delay field for that method.

4. If the delay sensor peak elutes BEFORE the MSD peak, determine the desired delay for the delay sensor. For example:

- LC flow = 10 ml/min
- Delay sensor = 1.0 min, MSD = 1.1 min
- Target time for delay sensor = 1.2 min or 0.2 min added delay.
- Cut appropriate length of supplied 1/16" od x 0.04" id tubing to get this delay. Tubing yields 8.1 ul/cm, so 0.2 min @ 10 ml/min = 25 cm more tubing length.

5. Rerun the delay sensor calibration to recalibrate. Enter the new delay time into the collector delay field. If an 1100 detector is also present, select the Calibrate button to establish the new delay volume.

Mass-based fraction collection checkout

Follow the instructions given in the "User's Guide for Purification/High Throughput Systems" (p/n G2262-90001) to perform the checkout of the mass-based fraction collection system. Electrospray LC demo sample (p/n 59987-20033) and a Zorbax SB-C18 checkout column is provided in the mass-based fraction collection kit. The column should be installed between the sampler and the LC detector or the sampler and the splitter if there is no LC detector.

Splitter Maintenance

The maintenance for the splitter consists of replacing the rotor seal and stator face assembly every 1 million cycles. If the splitter is running constantly (24 hours/day), that corresponds to approximately two weeks/1 million cycles.

Follow the instructions given in the Rheodyne "MRA™ Operating Manual" supplied with the active splitter for information and procedures on valve maintenance.