

Installation Guide – Thermo Scientific[™] Vanquish[™] Duo for Inverse Gradient

Observe the safety information and precautionary statements, unpacking and installation instructions presented in the *Operating Manual* for the respective Thermo Scientific[™] Vanquish[™] modules.

This guide provides condensed information for installing a Thermo Scientific Vanquish Horizon Duo or Flex Duo UHPLC system for Inverse Gradient.

Parts required

Table 1: Modules required for installation of Vanquish Horizon Duo or Flex Duo UHPLC system for Inverse Gradient

Component	Vanquish Horizon Duo for Inverse Gradient			Vanquish Flex Duo for Inverse Gradient			
System: Base, Rack &		• 1x Syster	m Base Vanquish He	orizon/Flex (VF-S01-A-02)			
Capillaries		 1x Vanqu 	uish Duo for Inverse	e Gradient Kit (6036.2010)			
		Stack Stabi	lizer for stacks > 4 i	modules (optiona	<u>al)</u>		
		 Ion Bencl 	h with stack mount	ing kit (6036.172	20) or		
		 Bench Cla 	amp Kit for benchte	op installation (6	036.1740) or		
		 Stack Sta 	bilizer Kit for bench	ntop installation	(6036.1710)		
Pumps	• 2x Binary Pump H (VH-P10-A-02) <i>or</i>			 1x Dual Pump 	o F (VF-P32-A-01) <i>or</i>		
	• 1x Binary Pump H and 1x Quaternary Pump F			• 2x Binary Pump F (VF-P10-A-01) <i>or</i>			
					 2x Quaternary Pump F (VF-P20-A) or 		
				 1x Binary Pump F and 1x Quaternary Pump F 			
Samplers	1x Split Sampler HT (VH-A10-A-02)			1x Split Sample	er FT (VF-A10-A-02)		
Optional Sample Extension			Charger (VH-A90-A)			
Column Compartments		1x Column	Compartment H (V	′H-C10-A-02)			
Detectors	Charged Aerosol	Optional					
	• H (VH-D20-A)	Diode Array	Diode Array Fluorescence		Variable Wavelength		
	• F (VF-D20-A)	• HL (VH-D10-A)	• F (VF-D50-A)	1	F (VF-D40-A)		
		• FG (VF-D11-A-01)	• F Dual-PMT	(VF-D51-A)			
Flow Cells	n.a.		Selection of wi	de range of flow cells per each optical detector			





Figure 1: Flow Schemes Overview Vanquish Duo for Inverse Gradient

Q_6

Pump right







Table 2: Required system tubing and items, compatible with any configuration from Table 1.

No.	Description	No.	Description
1	Viper Capillary, 0.10 x 350 mm, MP35N (6042.2340) ²	6	Viper Capillary, 0.10 x 65 mm, MP35N (6042.2306) ¹
2	Active Pre-Heater, 0.1 x 380 mm, VH-C10 (6732.0110) ² optional: Passive Pre-Heater 0.1 x 530 mm, MP35N (6732.0174) ²	7	Viper Capillary, 0.10 x 950 mm, MP35N (6042.2395) ¹
3	Only used if optical detector is present ⁴ For two separate pumps: Post Cooler 1µL, 0.1 x 590 mm, VH-C10 (6732.0520) ¹	8	<i>Flow Schemes Vanquish Duo for Inverse Gradient</i> (4820.3615): four different flow schemes are included in the delivery showing the stack and tubing for four different configuration options. Flow Schemes can be clipped into right sampler door ¹
4	Viper Capillary, 0.10 x 350 mm, MP35N (6042.2340) ⁵		Optional: Set color marker labels (blue and red) for capillaries, solvent lines and wash lines ¹
5	Tee Piece ID 0.020" (0.5 mm) for $1/16$ " capillary (6263.0035) ¹		

¹ Included in Vanquish Duo for Inverse Gradient Kit (6036.2010), ² Included in System Base Vanquish Horizon/Flex (VF-S01-A-02), ³ not shipped with system, to be ordered separately, ⁴ Included in respective optical detector ship kit, ⁵ Included in Charged Aerosol Detector ship kit



Hardware and Software Installation

- 1. Locate and unpack all required system components as shown in Table 1 and Table 2.
- 2. Identify your configuration out of the four *Flow Scheme* pictures and stack the modules accordingly. Some Vanquish configurations may require the stacking of more than 4 modules (e.g. Vanquish Duo setups with two separate pumps). This installation mandatorily requires an appropriate protection against tilting.

NOTICE: Thermo Fisher Scientific offers various technical solutions for tilting protection, e.g. Bench Clamp Kit (6036.1740) or Stack Stabilizer Kit (6036.1710) for benchtop installation or Ion Bench with Stack Mounting Kit (6036.1720). Make sure that an appropriate tilting protection is installed for safe operation.

TIP: For installation instructions of the stack stabilizing follow the respective instructions delivered with the stack stabilizing options.

- 3. Connect all power cords, USB and system interlink cables as described in the respective modules *Operating Manual*. Diode array detectors may need to be plugged to the PC individually.
- 4. Connect all system tubing and solvents
 - a. For solvent lines follow the Vanquish Pumps Operating Manual.
 - b. For rear seal tubing follow the Vanquish Pumps and Sampler Operating Manual.
 - c. For needle wash tubing follow the Vanquish Sampler Operating Manual.
 - d. For system capillaries install each capillary according to the numbering on the *Flow Scheme* belonging to your configuration and part of the deliverd capillary kit. Instructions on how to install the passive pre-heater are outlined in the *Installation Note* of the passive pre-heater. For installing the capillaries correctly, please read the *Viper Installation and Operating Guide*.

TIP: The *Flow Scheme* of the installed system configuration can be clipped into the right sampler door.



Figure 2:Flow scheme in sampler door



- 5. Optionally install tubing markers that are delivered with the Vanquish Duo system ship kit on the flow paths for optical distinction.
- 6. Install the columns.

TIP: If operational qualification of the hardware is required please follow the *Operating Instructions for Operational Qualification/Performance Qualification for HPLC Instruments.*

- 7. Switch on all modules in the system.
- 8. Install the Thermo Scientific Chromeleon 7.2.8 (or higher) Chromatography Data System (CDS). Follow the instructions of section Software Configuration to configure and operate the Vanquish Duo for Dual LC system.

Software Configuration

- 1. Open the **Chromeleon Services Manager** and start the **Instrument Controller** (if not started automatically on system start).
- 2. Open the Chromeleon Instrument Configuration Manager and create one Instrument



Figure 3: Instrument in Chromeleon

- 3. Add two pumps to the instrument
 - a. If a Dual Pump is present choose HPLC: Vanquish followed by Vanquish Dual Pump. The left and right pump are automatically assign to the previously generated instrument on the Devices page. Ensure that the device names for the instrument devices are not identical (e.g., PumpLeft and PumpRight). It is recommended to add a descriptive extension to the respective device names and signal names. This will show up on the module panels and simplifies distinction.



Dual Pump Configuration	1	🗐 Dual Pi	ump Conf	iguration		×
Signals Outputs Inputs General Devices Left Limits Left Solvents Right Limits Right Solvents Bottles		General Signals	Devices Outputs	Left Limits	Left Solvents Right Limits Ri	ght Solvents Bottles
Main Device			Enabled	Туре	Name	Factor
Name, prompriseduo				Pressure	PumpLeft_Inv_Pressure	1.000
		+	~	Pressure	PumpRight_Ana_Pressure	1.000
Name Instrument PumpLeR_Inverse Right Pump Device Name Instrument PumpRight_Analytical InverseGradient						
OK Cancel Help						ancel Help

Figure 4: Dual Pump configuration

b. If two separate pumps are present choose **HPLC: Vanquish** followed by the respective pump type. It is recommended to add a descriptive extension to the respective device names and signal names. This will show up on the module panels and simplifies distinction.

Vanquish Binary Pump Configuration	Vanquish Binary Pump Configuration	×
General Devices Limits Solvents Signals Outputs Inputs	General Devices Limits Solvents Signals Outputs Inputs	
Main Device Name: PumpModule_Upper_Inverse	Enabled Type Name Factor	
	▶ IV Pressure Pump_Up_Inverse_Pressure 1.000	
Pump Device Name		
Pump_Upper_Inverse		
OK Cancel Help	OK Cancel Help	,

Figure 5: Two pumps configuration

4. Add the Sampler to the instrument. Choose **HPLC: Vanquish** followed by **Vanquish Autosampler**. Assign the appropriate pump links if applicable (e.g., *VPUMP_R_STRK* for the default Dual Pump configuration). Add a Vanquish Charger module if present in the configuration.



✓ Enable Sir	nulation Mode Module Address:	VF-A10-A Browse
Charger	COM Port	Try Connect
^p ump Link -	Flow through sampler is delivered by pump	
PUMP_R_S	TRK (Vanquish Dual Pump)	•

Figure 6: Sampler configuration with Dual Pump

- 5. Add the Column Compartment to the instruments. Choose **HPLC: Vanquish** followed by **Vanquish Column Compartment**.
- 6. Add the detector to the instrument. Choose **HPLC: Vanquish** followed by the respective detector type.
- 7. Save the configuration and launch Chromeleon.



Figure 7: Final instruments configuration

8. Select one instrument and launch the Fluidic Configuration wizard.

Fluidic Configuration Wizar	rd	
Back Create File Edit View Tools	Help	
Instruments ≪ ■ ○ ☆ Filter ▼	→ Launch eWorkflow → 🤤 → 👔 Take Control Home Pump_Lower_Analytical Pump_U	yper_Inverse Sampler UV CAD Audit Startup Queue
The local Instrument Controller is running idle. Stop local Instrument Controller.	nverseGradient	Injection:
DEGER-BTSRF82	/anguish Charged Aerosol Detector	

Figure 8: Fluidic configuration in Chromeleon

9. On the first wizard page select the installed capillary kit from the drop down list, here **Vanquish Inverse Gradient**.



Define Fluidic Configuration	0
Select Fluidic Configuration Please select the capillary kit or the instrument.	fluidic configuration for the
Fluidic Configuration (Capillary Kit)	Vanquish Inverse Gradient 💌
	Import Description From File
	Next >> Cancel

Figure 9: Workflow selection in Fluidic Configuration

TIP: The drop down list entries are automatically tailored to the previously created hardware configuration.

 Verify correct assignment of fluidic items, e.g. is the column in use connected to the left or right active pre-heater, and click Next. If columns are not configured in the Vanquish Column Compartment Instrument Configuration they will not show up in the list.

De	fine Fluidic Configuration		🔘 🗙	De	fine Fluidic Configuration	@ ×
As	sign Modules Please select the correct Chrom	ieleon device for each fluidic item.		As	sign Modules Please select the correct Chror	neleon device for each fluidic item.
	Fluidic Item	Chromeleon Device			Fluidic Item	Chromeleon Device
►	Inverse Gradient Pump	PumpModule.PumpLeft_Inverse	▼ ²	►	Inverse Gradient Pump	PumpModule_Upper_Inverse.Pump_Upper_Invers •
	Analytical Pump	PumpModule.PumpRight_Analyt	*		Analytical Pump	PumpModule_Lower_Analyt.Pump_Lower_Analyti -
	Pre-heater Capillary	ColumnComp.PrehtLeft	-		Pre-heater Capillary	ColumnComp.PrehtLeft -
	Column	ColumnComp.Column_A	•			
<u> </u>						
		<< Back Next >>	Cancel			<< Back Next >> Cancel

Figure 10: Assignment of fluidic items

- 11. Define the column volume.
 - a. If the columns are configured in the Vanquish Column Compartment Instrument Configuration and the written column tag is used the column parameters are filled and calculated automatically. Click Finish.



Define Fluidic Configuration Define Column Volume The Column ColumnComp.Colum contains the following information	n_A is described by an	n ID tag which	× @
Column Parameters			
Length	150.00	[mm]	
Inner Diameter:	2.10	[mm]	
Column Volume			
Effective Void Volume:	360.0	[µl]	

Figure 11: Automated fill of column information

b. If the columns are not configured in the Vanquish Column Compartment Instrument Configuration or no column tag is used the column parameters need to entered and the void volume will be calculated. If the column void volume was experimentially determined select column volume and enter the value accordingly Click Finish.

$$V = \pi * \frac{d_c^2}{4} * L * \varepsilon_T$$

V: Column Void Volume [μL] d_c: Column Diameter [mm] L: Column Length [mm] ε_T: Total porosity (0.63)

Define Fluidic Configuration	() x	Define Fluidic Configuration	() x
Define Column Volume Enter the column inner diameter and length or Column.	r the effective volume of the	Define Column Volume Enter the column inner diameter and Column.	length or the effective volume of the
Column Parameters		Column Parameters	
Length	[mm]	Length	100.00 [mm]
Inner Diameter:	[mm]	Inner Diameter:	2.1 [mm]
Calculated Void Volume:	[µ]	Calculated Void Volume:	218.2 [μ]
💿 Column Volume		🔿 Column Volume	
Effective Void Volume:	[µ]	Effective Void Volume:	[lu]
	<< Back Finish Cancel		Kack Finish Cancel
	Define Fluidie Configuration	<u> </u>	~
	Define Column Volume Enter the column inner diameter and length or Column.	r the effective volume of the	
	Column Parameters		
	Length	[mm]	
	Inner Diameter:	[mm]	
	Column Volume		
	Effective Void Volume: 220.2	[µ]	
		<< Back Finish Cancel	

Figure 12: Manual column definition



12. Check the Audit Trail if the Fluidic Configuration was saved accordingly.

	Date	Time	Retention Time	Device	Message
7	3/16/2018	1:10:02 PM +01:00			The capillary kit / application Vanquish Inverse Gradient' has been selected.

Figure 13: Audit trail message from fluidic configuration

- 13. To create an instrument method click **Instrument Method...** in the **Create** drop down menu.
- 14. Select Inverse Gradient on the Workflow Selection page. Click Next.



Figure 14: Workflow Selection Page

15. On the **Inverse Gradient Options** page select the desired **Calculation mode**. The Inverse gradient offset is automatically filled form the fluidic configuration taking the following volumes into account: System capillaries from installed kit, pump mixers, sampler fluidics, flow cell volumes. If the offset was determined by the user the value can be entered here overwriting the calculation from the fluidic configuration.

thermo scientific

📸 Instrument Method Wizard - In	nverse Gradient: Options
Use this option if you want to compe conditions at the detector. The inver	ensate the analytical gradient with an inverse gradient in order to get isocratic regradient will then be calculated automatically.
Analytical pump:	Pump_Lower_Analytical
Inverse gradient pump:	Pump_Upper_Inverse
Inverse gradient offset:	40513 Q [0.0010000.00 μ]
Calculation mode:	Keep solvent composition (recommended)
Maximum detector flow:	2.000 (0.00010.000 ml/min)
	< Back Next > Cancel Help

Figure 15: Inverse Gradient Options Page

16. Enter the analytical gradient in the gradient table on the Inverse Gradient: Flow Gradients page. The inverse gradient is automatically generated based on the previously selected Calculation Mode and is therefore here read only. To see the generated gradient table of the inverse gradient either the gradient image or the table tab can be clicked.



Figure 16:Inverse Gradient Flow Gradients Page



17. If the gradient or the calculation mode needs to be adapted this can be done at any time in the method editor under Inverse Gradient (Flow Gradients) and Inverse Gradient (Options). The user interface is similar to the method wizard.



Figure 17: Method Editor of Inverse Gradient Method

18. Create a sequence by clicking **Sequence...** in the **Create** drop down menu and add the Inverse Gradient instrument method to start analysis.