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# 1 Introduction

## Read this before setup



The instructions in this document provide information that is important for safety and functionality of the I/O-box-based connection of multiple lab scale unit operations. Read the entire document carefully before setting up the I/O-box-based connection of multiple unit operations.

## Purpose of the instructions

It is possible to utilize I/O boxes when connecting unit operations in your downstream purification process. The unit operations can consist of different constellations of ÄKTA™ chromatography systems, stand alone sensors, pumps etc.

This document provides trained personnel with the instructions needed to set up the I/O-box-based connection for the lab scale unit operations in a safe way. The I/O-box-based connection setup described in this document should only be viewed as an example. This document should serve as a help and guidance for an operator when setting up the I/O-box-based connection of multiple unit operations.

## Scope of the instructions

This document describes the following:

- Examples of lab scale unit operations that can be connected using I/O-boxes
- How to connect unit operations using I/O-boxes
- Function of the unit operations in an example setup

This document does not include the following information:

- How to install the components that are part of the unit operations at the laboratory site.
- How to operate the components that are part of the unit operations at the laboratory site.

**Note:** *For information about installing, cleaning, operating or storing the systems used for the unit operations, refer to the respective system Operating Instructions, or the user documentation for the appropriate accessory.*

## Typographical conventions

Software items are identified in the text by ***bold italic*** text.

Hardware items are identified in the text by **bold** text.

In electronic format, references in *italics* are clickable hyperlinks.

## Notes and tips

**Note:** *A note is used to indicate information that is important for trouble-free and optimal use of the product.*

**Tip:** *A tip contains useful information that can improve or optimize your procedures.*

## Abbreviations

The following table defines the meanings of abbreviations and acronyms used in these instructions.

Abbreviation/acronym	Definition
4C	4 column
BSA	Bovine serum albumin
CIP	Cleaning in place
PCC	Periodic Counter Current

## 2 Safety instructions

The user must have read and understood the Safety instructions chapter for all the systems and accessories that are part of the unit operations in the I/O-box-based connection setup.

For detailed safety instructions, refer to the Operating Instructions or risk analysis for the individual system or accessory.

# 3 Description of the unit operations

## Introduction

This chapter describes the unit operations and the I/O-box.

The types of unit operations described here are only intended as examples.

## Unit Operations

Type of unit operation	Components
Simulated perfusion cell culture unit	Peristaltic pump
	BSA solution (Simulated perfusion cell culture solution)
	Scale with analog out connector
ÄKTA pcc unit	ÄKTA pcc system (in 4C bind and elute configuration)
	I/O-box <sup>1</sup>
	<b>Analog in/out</b> and <b>Digital in/out</b> connector with screw terminal
Simulated virus inactivation unit	pH meter with analog out
	Acid solution
	Base solution
	Magnetic stirrer
	Peristaltic pump with remote controll capability (2 pcs)
	Flask for pH adjustment and measurement
ÄKTA pure unit	ÄKTA pure system
	I/O-box <sup>1</sup>
	<b>Analog in/out</b> and <b>Digital in/out</b> connector with screw terminal

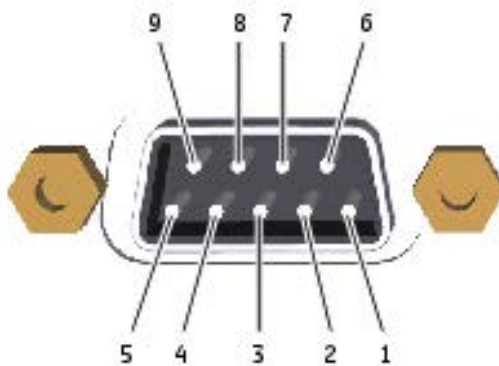
<sup>1</sup> Make sure the remote control protocol must match the I/O-box specifications.

## Connectors on I/O-box



Part	Description
<b>Analog in/out</b>	Signal connector for analog input and output signals.
<b>UniNet-9</b>	Connector used to connect the I/O-box to the instruments.
<b>Status</b>	Status indicator for service purposes.
<b>Node ID</b>	Switches used to configure <b>I/O-box</b> as I/O-box or I/O-box, 2nd.
<b>Digital in/out</b>	Signal connector for digital input and output signals.

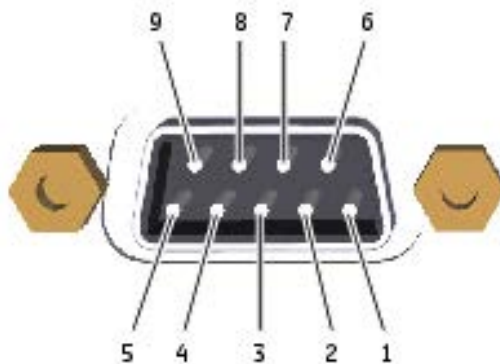
## Analog connector pins



Part	Function
1	Analog in signal 1 +
2	Analog in signal 1 - (or signal ground)

Part	Function
3	Shield, analog in (both ports)
4	Analog in signal 2 +
5	Analog in signal 2 - (or signal ground)
6	Calibration pin for service purposes Analog out signal (1.9 V) <b>Note:</b> <i>Do not use for other purposes.</i>
7	Analog out signal 1
8	Signal ground, analog out (both ports)
9	Analog out signal 2

## Digital connector pins



Part	Function
1	Digital in signal 1
2	Digital in signal 2
3	Digital in signal 3
4	Digital in signal 4
5	Signal ground



Part	Function
6	Digital out signal 1
7	Digital out signal 2
8	Digital out signal 3
9	Digital out signal 4

## Connectors on ÄKTA pcc

The illustration below shows where the connectors are located on the ÄKTA pcc instrument. For connectors on the computer equipment, refer to the manufacturer's documentation.

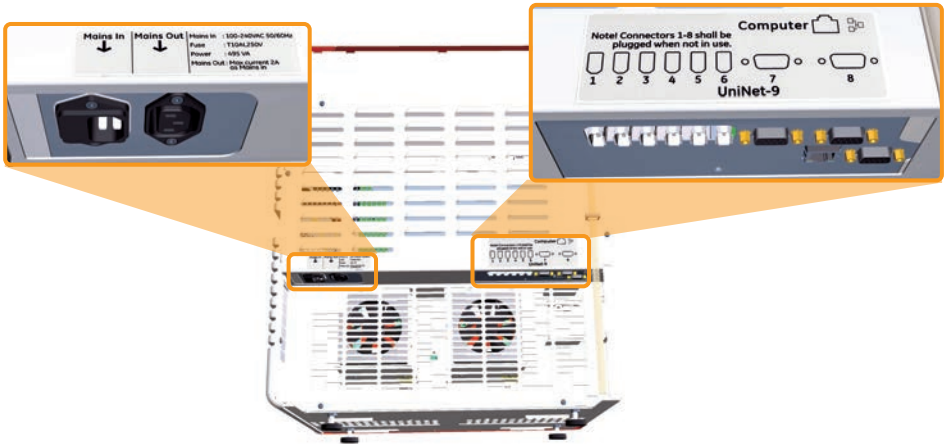


Part	Function
1	<b>Power</b> input connector
2	<b>Network</b> connector (Ethernet)
3	<b>UniNet-9</b> connectors  <b>Note:</b> <i>Termination plugs must be connected to the connectors that are not in use.</i>

Other connectors are for use by authorized service engineers only.

## Connectors on ÄKTA pure

The illustration below shows where the connectors are located on the ÄKTA pure instrument. For connectors on the computer equipment, refer to the manufacturer's documentation.



# 4 Setup

## Introduction

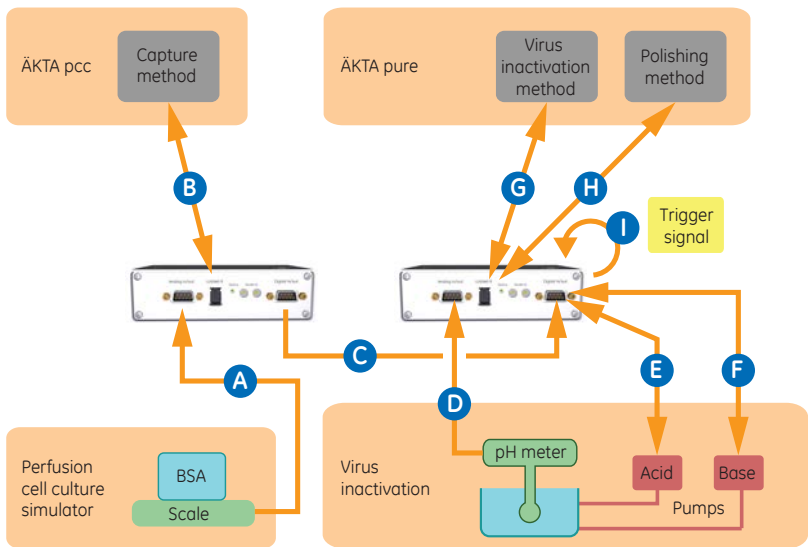
This chapter describes an example setup of an I/O-box-based procedure to connect multiple single unit operations and how data will flow in the between them.

For a detailed description of the ÄKTA pcc and ÄKTA pure systems, refer to *ÄKTA pcc Operating instructions 29114809* and *ÄKTA pure Operating Instructions 29022997*, respectively.

For a detailed description of the I/O-box, refer to *Install I/O-box E9 Instructions 29021463*.

## I/O-box-based connection chart and dataflow

Connect the unit operations according to the image below:



The dataflow between the unit operations depend on the UNICORN™ methods that run on the ÄKTA pcc and ÄKTA pure systems.

The unit operations in this setup can run three different types of UNICORN methods, **Capture**, **Virus inactivation**, and **Polishing**.

The table below describes dataflow between the connected unit operations.

**Note:** Use a **Analog in/out** and **Digital in/out** connector with screw terminal, to connect to individual Analog or Digital connector pins.

Components	Dataflow
Upstream or scale to <b>Capture</b> method	<ol style="list-style-type: none"> <li data-bbox="543 151 1019 263">1. The scale sends the measured weight data as an analog out signal to the Analog in pin in the <b>Analog in/out</b> port on the ÄKTA pcc I/O-box (A).</li> <li data-bbox="543 279 1019 367">2. The <b>Capture</b> method (on the ÄKTA pcc system) receives data from the ÄKTA pcc I/O-box through a <b>UniNet-9</b> connector (B).</li> </ol>
<b>Capture</b> method to <b>Virus inactivation</b> method	<ol style="list-style-type: none"> <li data-bbox="543 402 1019 489">1. The <b>Capture</b> method (on the ÄKTA pcc system) sends data to the ÄKTA pcc I/O-box through a <b>UniNet-9</b> connector (B).</li> <li data-bbox="543 505 1019 617">2. The data is transferred from the ÄKTA pcc I/O-box to the ÄKTA pure I/O-box from a Digital out pin to a Digital in pin in the <b>Digital in/out</b> ports (C)</li> <li data-bbox="543 633 1019 721">3. The <b>Virus inactivation</b> method (on the ÄKTA pure) receives data from the ÄKTA pure I/O-box through a <b>UniNet-9</b> connector (G)</li> </ol>
pH meter to <b>Virus inactivation</b> method	<ol style="list-style-type: none"> <li data-bbox="543 753 1019 865">1. The pH meter sends the measured pH data as an analogue out signal to an Analog in pin in the <b>Analog in/out</b> port on the ÄKTA pure I/O-box (D).</li> <li data-bbox="543 880 1019 992">2. The <b>Virus inactivation</b> method (on the ÄKTA pure system) receives data from the ÄKTA pure I/O-box through a <b>UniNet-9</b> connector (G).</li> </ol>
<b>Virus inactivation</b> method to pumps	<ol style="list-style-type: none"> <li data-bbox="543 1035 1019 1147">1. The <b>Virus inactivation</b> method (on the ÄKTA pure system) sends data to the ÄKTA pure I/O-box through a <b>UniNet-9</b> connector (G).</li> <li data-bbox="543 1163 1019 1275">2. The ÄKTA pure I/O-box sends data to the peristaltic pumps through two different digital out pins in the <b>Digital in/out</b> port (E and F).</li> </ol>

Components	Dataflow
<p><b>Virus inactivation</b> method to <b>Polishing</b> method</p>	<p>1. The <b>Polishing</b> method is started by a Trigger signal. The value of the Trigger signal is set in the UNICORN instruction and act as a signal activating a watch block.</p> <p><b>Note:</b></p> <p><i>A trigger signal is created by connecting the Digital out pin to the Digital in pin of the <b>Digital in/out</b> connector on the same I/O-box. For more information, see <a href="#">Connectors on I/O-box, on page 7</a>.</i></p>

# 5 Functionality

## Introduction

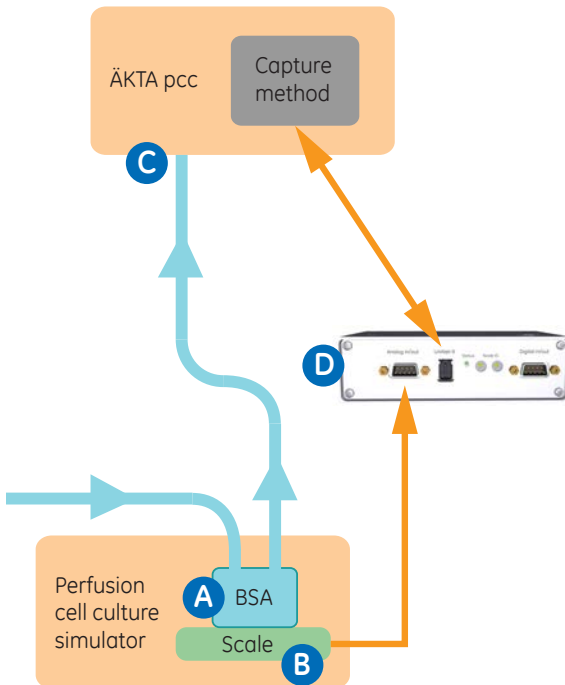
This chapter describes the functionality and communication between the connected unit operations.

The components of each unit operation are described in [Unit Operations, on page 6](#).

The set up of the I/O-box-based connection of multiple unit operations is described in [I/O-box-based connection chart and dataflow, on page 11](#).

## Simulated perfusion cell culture

Simulated perfusion culture is described below.



Part	Function
A	A peristaltic pump delivers a constant flow of sample (BSA solution) to a hold up tank. This setup simulates perfusion cell culture.

Part	Function
B	A hold up tank that stands on a scale. The scale measures the amount of sample in the hold up tank.
C	Sample is delivered to the ÄKTA pcc system through one of the system inlets.
D	The scale communicates with the ÄKTA pcc system through the ÄKTA pcc I/O-box.

## Communication between the scale and the *Capture* method

As long as the amount of sample in the hold up tank is below what is needed to run one full loop, the ÄKTA pcc system is in **Hold** state.

When enough sample for running one loop has been collected in the hold up tank, a signal from the scale will trigger the ÄKTA pcc system to continue the **Capture** method and run one loop.

One loop in the ÄKTA pcc **Capture** method corresponds to: capture, wash, elution, CIP, and equilibration on one of the system's four columns.

**Tip:** *A setup without a hold up tank can be used. This can reduce time of the method and minimize risk of infection and sample degradation. However, a setup without a hold up tank can amplify the impact of errors and can be challenging to accomplish with potential fluctuations in flow rate.*

**Tip:** *Different signals such as weight of sample, level of sample, or time can be used to trigger the ÄKTA pcc system.*

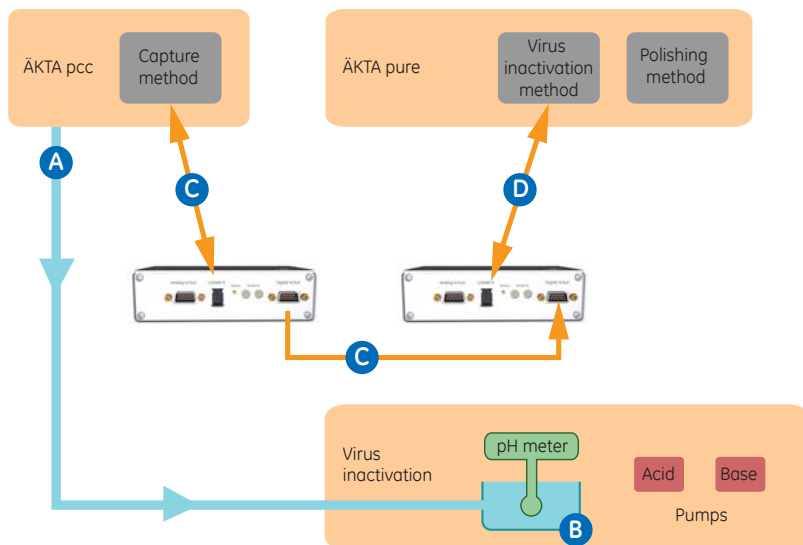
## Modification of *Capture* method

The **Capture** method used in these instructions can be modified as described below:

- Watch-blocks can be added to the **Capture** method to monitor Analog in signals. This lets the method execute steps depending on the amount of sample in the hold up tank.
- A Digital out signal can be added to signal to the **Virus inactivation** method when an elution step has taken place.

## Communication between the **Capture** method and the **Virus inactivation** method

The communication between the **Capture** method and the **Virus inactivation** method is described below.



Part	Function
A	The elution fractions from the ÄKTA pcc containing the target protein are pooled.
B	The pooled fractions are collected in the virus inactivation reservoir where pH adjustment takes place.
C	After each fraction containing the target protein is collected, a signal is sent from the ÄKTA pcc system to the ÄKTA pure I/O-box.
D	The <b>Virus inactivation</b> method running on the ÄKTA pure system counts the fractions and initiates the <b>Virus inactivation</b> method at a set number.

**Tip:** The **Virus inactivation** method can be set to start after one or several completed loops of the **Capture** method.

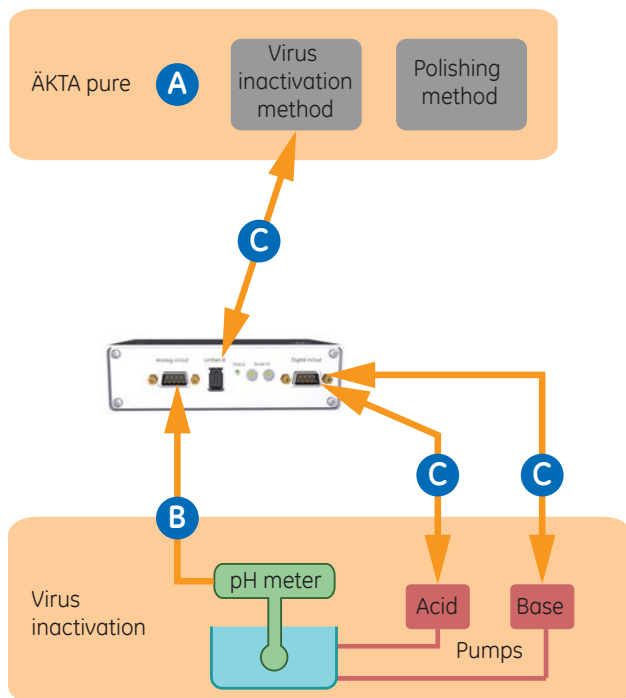
**Tip:** The sample can be stored before it is transferred to the virus inactivation reservoir provided the sample itself is stable enough.



**Note:** The pH meter must have an Analog out that can be connected to the ÄKTA pure I/O-box.

## Virus inactivation method

The **Virus inactivation** method is described below.

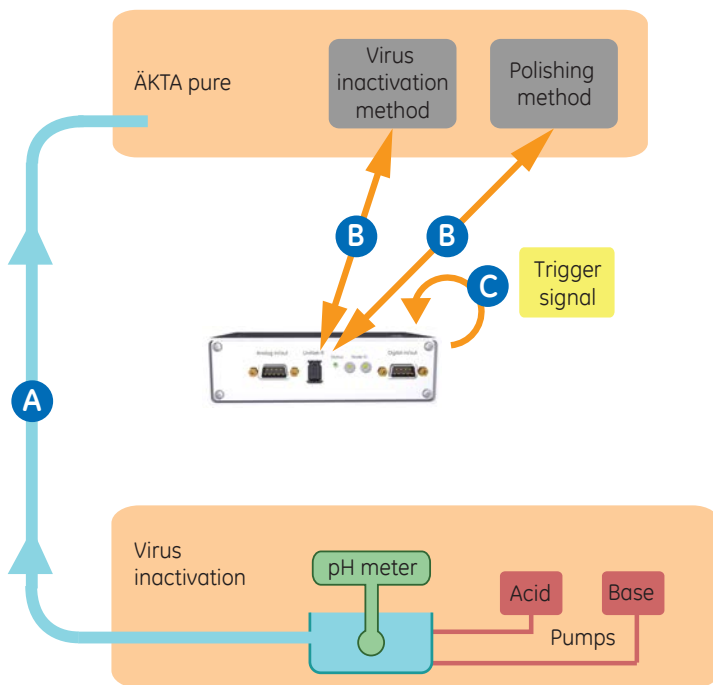


Part	Function
A	The two methods, the <b>Virus inactivation</b> method and the <b>Polishing</b> method, can run simultaneously on one ÄKTA pure system
B	The <b>Virus inactivation</b> method monitors the pH in the virus inactivation reservoir.
C	The <b>Virus inactivation</b> method controls the addition of the acid and base solutions to the virus inactivation reservoir, through the two pumps.

**Tip:** It is possible to run the two UNICORN methods, the **Virus inactivation** method and the **Polishing** method, on the same system by merging them into one UNICORN method where **Virus inactivation** and **Polishing** can act independently from each other. The resulting new method must be progressed by watch block triggered from specific events, and not time or volume. This is achieved by setting all instructions to **breakpoint zero**. The time for virus inactivation was instead monitored with an external timer connected to the **Digital in/out** of the I/O-box belonging to the ÄKTA pure system.

## Communication between the **Virus inactivation** method and the **Polishing** method

The communication between the **Virus inactivation** method and the **Polishing** method is described below.



Part	Function
A	The sample is loaded from the virus inactivation reservoir to the ÄKTA pure system through a sample inlet.
B	A trigger signal starts the <b>Polishing</b> method when the <b>Virus inactivation</b> method sequence is finished.
C	The trigger signal is created by connecting the Digital out pin to the Digital in pin of the <b>Digital in/out</b> connector on the same I/O-box. For more information, see <a href="#">Connectors on I/O-box, on page 7</a> .

**Tip:** *Instead of a trigger signal, the **Polishing** step can be activated after the **Virus inactivation** method, by placing the method in the method queue.*

**Note:** *The **polishing** method can be started directly after the **Virus inactivation** method. Alternatively, the **Polishing** method can run in parallel with the **Virus inactivation** method.*

## Flow path modifications for the **Polishing** method

The flow path for the **Polishing** method that run on the ÄKTA pure system can be modified as described below:

- An air sensor can be added to the inlet tubing between the virus inactivation reservoir and the system inlet. This lets the method stop the sample loading when the virus inactivation reservoir is empty and air enters the tubing.

**Note:** *Before starting sample loading through the inlet tubing, a small volume can be pumped backwards to remove the air from the tubing.*

- A T-connector can be added to the tubing between the sample pump and the sample inlet valve.
- Tubing from an outlet valve can be connected to the T-connector. This lets the system direct the flow to refill the inlet tubing.

## 6 Reference Information

For detailed reference information, refer to the Operating Instructions and specifications for the individual system or accessory.

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