

Operator's Manual



Rev 1/22/2024

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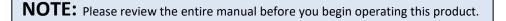
1. Introduction

Congratulations on your purchase of the ANKOM^{FLEX} Analyte Extractor (FLEX). We are confident that this product will effectively serve your needs.

The FLEX is designed to simplify fat-soluble vitamin, cholesterol, crude fat, and total (hydrolysis) fat determinations. Supporting up to four samples at a time, the system will automatically complete digestion (saponification or hydrolysis), solid phase extraction (SPE), and evaporation of solvent in about two to three hours. By automating these labor-intensive procedures, accuracy and precision is improved, chemical exposure is limited, and hands-on labor is reduced. This manual will provide you with details that will help you operate the FLEX efficiently and effectively for precise and accurate results.

ANKOM Technology designs, manufactures, and markets analytical instruments and support products to laboratories around the world in the food, feed, agricultural, bioenergy, and environmental industries. Our instruments allow for the analysis of fat-soluble vitamins, cholesterol, dietary fiber, crude and detergent fiber, crude and total fat, digestibility, microbial fermentation (anaerobic or aerobic) and more.

ANKOM Technology is committed to total customer satisfaction, designing every product based on a thorough assessment of customer needs.



2. Warranty

ANKOM Technology warrants the ANKOM^{FLEX} Analyte Extractor against any defects in workmanship or material for one year after the original date of purchase. This warranty does not include damage to the instrument resulting from neglect or misuse. During the warranty period, should any failure result from defects in workmanship or materials, ANKOM Technology will, at its discretion, repair or replace the instrument free of charge. Extended warranties are available upon request.

3. Contact Information

We are committed to supporting you and value your feedback.

For any questions or suggestions regarding your instrument, please contact us at:

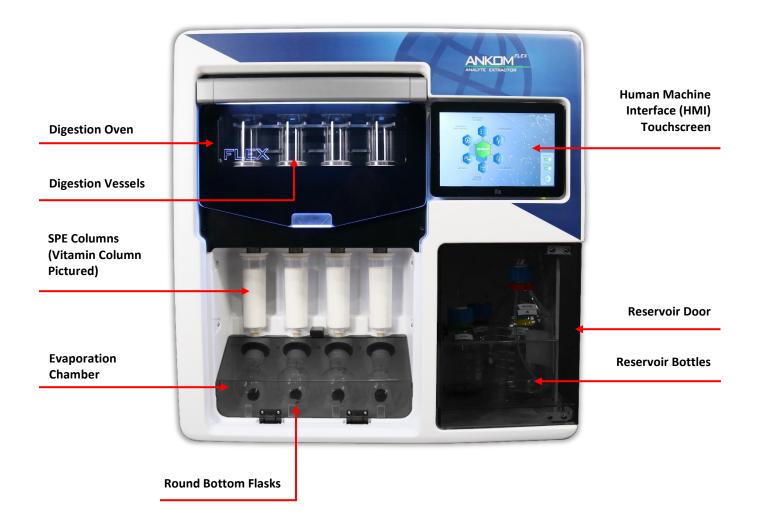
For Sales Support: <u>sales@ankom.com</u> or <u>https://www.ankom.com/contact-us</u> For Technical Support: <u>www.ankom.com/contact/technical-services</u> For Analytical Support: <u>www.ankom.com/contact/analytical-services</u> Telephone: (315) 986-8090 Fax: (315) 986-8091



4. Instrument Description

The ANKOM^{FLEX} Analyte Extractor simplifies fat-soluble vitamin, cholesterol, crude, and total (hydrolysis) fat determinations and other possible applications. This fully automated system integrates all the steps involved in these analyses in one instrument. Supporting up to four samples at one time, the system will automatically complete digestion (saponification or hydrolysis), solid phase extraction and evaporation of the solvent. The system has four solution reservoirs which can be filled with various chemical solutions as defined by method requirements (e.g. ethanol, hexane, HCl, KOH, water). The FLEX's software allows for method development and customization.

Digestion vessels have been designed to allow for sample to be weighed directly into the vessels, thereby eliminating transfer error. Filtration takes place without user intervention, further reducing technician labor. A nitrogen atmosphere, positive flow, and low temperature evaporation protects sensitive analytes against oxidation and optimizes solvent evaporation. Exhaust lines can be connected to an exhaust system therefore eliminating the need to position the entire instrument in a ventilation hood. Once an assay is complete, the isolated analyte is ready for gravimetric analysis (fat analysis) or reconstitution for quantitation by HPLC or GC (vitamin, cholesterol, fatty acid profile analysis).





5. Safety Precautions

KLIM

ANALYTE EXTRACTOR

FLEX

Hot Surfaces – Do NOT open the Digestion Oven during operation. The interior surfaces can reach 100°C (212°F). Failure to observe this caution may result in burns.
Hazardous Voltages – Do NOT operate the instrument with the back of the main electrical cabinet removed. Hazardous voltages are present during operation. Failure to observe this caution may result in electrical shock or electrocution.
Hazardous Materials – Follow SDS warnings and recommendations for all materials to be used in this instrument, for example: Ethanol, Hexane, and SPE sorbent. Follow both local and federal regulations for vent hood requirements when operating this instrument. Failure to observe this caution may be hazardous to your health.
WARNING: Attempts to override safety features or to use this instrument in a manner not specified by ANKOM Technology voids the warranty and may result in serious injury or even death. This system is designed to meet and/or exceed the applicable standards of CE and CSA.

IMPORTANT: The power switch must be in the OFF position before plugging the instrument power cord into the power source.

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6. Instrument Installation

6.1 Site Requirements and Operating Environment

To install and operate the ANKOM^{FLEX} Analyte Extractor you will need the following:

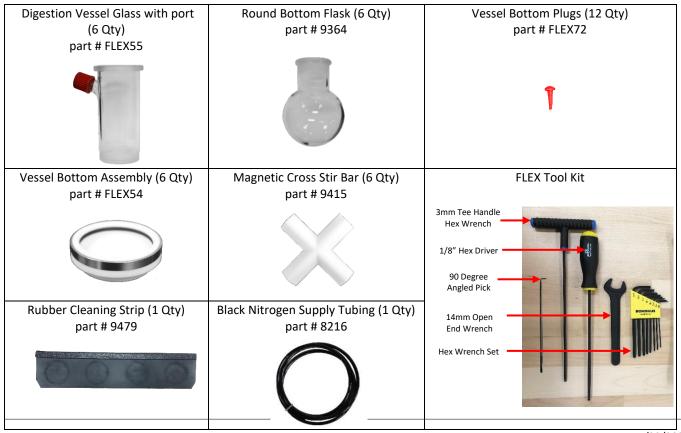
- Adequate Power: 100V–120V ~ 50/60Hz 8A
 - 220V–240V ~ 50/60Hz 8A
- Must have ability to connect to an exhaust system
- Bench space that can accommodate 77.5 cm (30.5") W x 81.3 cm (32") H x 45.7 cm (18") D and a weight of 54.4 kg (120 lbs.)
 - Nitrogen Supply Requirements: a. N2 purity, Industrial Grade (Grade 4.8) which is 99.998% or purer
 - b. N2 Flowrate of 10LPM at 6.9 bar minimum
 - c. Supply N2 pressure 5.5-6.9 bar (80-100 psi)
- Fitting adapter to connect nitrogen supply to the ¼" (6.35mm) outside diameter
- Black polyurethane nitrogen supply tubing that ANKOM supplies
- Ambient Temperature Range: 19°–30°C (66°-86°F)

6.2 Unpack Instrument

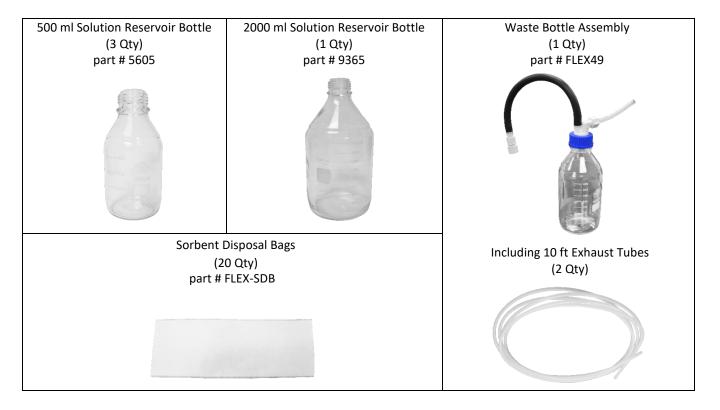
The instrument shipping container consists of three separate cardboard pieces: a top, a bottom, and a sleeve that forms the body of the container. Remove the top and the sleeve of the instrument shipping container. Lift the instrument from the shipping container bottom and place it on a surface that is firm and level. The instrument needs to be near a nitrogen supply and within range to connect exhaust lines to a ventilation hood or exhaust system. The instrument must not be subjected to excessive shock, vibration, dirt, moisture, oil, or other fluids.

6.3 Unpack Attachments and Accessories

Within the shipping container is a cardboard box containing attachments, accessories, and a tool kit. Open the box and verify that the following items are present. The tool kit will have a separate packing list included.







FLEX Support Items

The following support items can be purchased separately.

Item	ANKOM Part #
Vitamin & Cholesterol SPE Column	FLEX-SPE-01
Total Fat SPE Column	FLEX-SPE-02
Column Top	FLEX-9001
SPE-02 Container	FLEX-9002
Vitamin & Cholesterol Filter	FLEX-VF
Total Fat Filter	FLEX-FF
Cellulose Filter	FLEX-CF
Sorbent Disposal Bag	FLEX-SDB
FLEX Maintenance Kit	FLEX66

6.4 Wipe Components

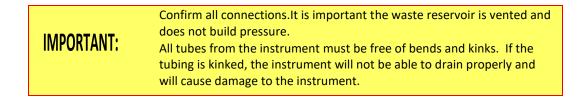
6.4.1. Wipe the vessel bases, the spray nozzles and O-rings of the digestion vessel tops and the column top adaptors with a towel wetted with water. Refer to FLEX Service Procedure- End of Run Cleaning (FLS011).

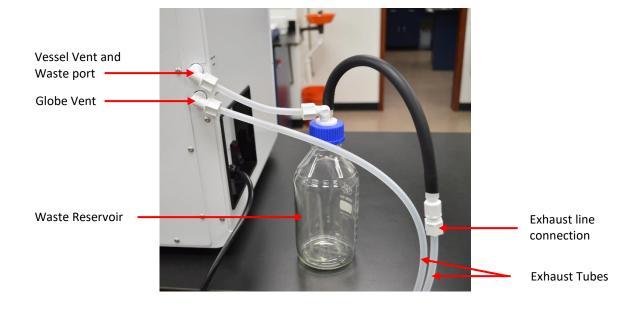




6.5 Make Connections

- 6.5.1. Connect waste bottle assembly. If your laboratory would like to connect the instrument to its own waste reservoir, be sure to adhere to the standards below.
 - Connect the shorter opaque tube on the waste bottle assembly to the vessel vent and waste port on the back of the instrument. The instrument **cannot** drain uphill; it must drain by gravity. This means that the waste reservoir **cannot** be higher than the waste port. This scenario would create a backflow to the instrument.
 - Connect one of the 3 m exhaust tubes to the globe vent port on the back of the instrument.
 - Connect the second 3 m exhaust tube to the waste assembly exhaust line connection.
 - Vent the exhaust tubes to an exhaust system. The exhaust system should pull a slight vacuum so that solvent fumes are properly removed from the FLEX during evaporation. These connections must never be closed or restricted during operation of the instrument.
 - Screw on the cap to the waste reservoir.



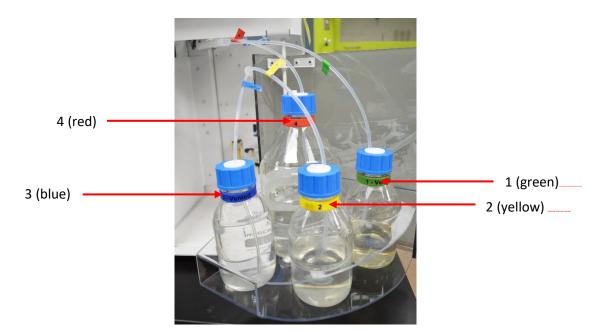




6.5.2. Connect nitrogen supply (N₂ high pressure) - Attach the black nitrogen supply tubing (part # 8216) to a high pressure (>50psi) nitrogen source and then attach to the instrument by pushing the black nitrogen supply tubing into the nitrogen supply port on the back of the instrument. Refer to FLEX Service Procedure – Attaching & Detaching Nitrogen (FLS002), located on the ANKOM website.



6.5.3. Press the reservoir door to open the door. Install the solution reservoir bottles into the correct numbered position. Match the colors and numbers of the reservoir bottles to each reservoir cap and tube.





6.6 Power On

6.6.1. Plug the electrical cord into an appropriate power source.

6.6.2. To turn on the FLEX, toggle the power switch that is located on the side of the instrument. The red light indicates that it is on.



Electrical Cord

The FLEX will take about three minutes to power on and run a self-test. The self-test will conclude, and the following screen will be displayed.

If the pressure sensors do not pass, press "OK" and proceed to the following section to adjust the nitrogen pressure regulators.

If any other component does not pass the self-test, contact ANKOM. www.ankom.com/contact/technicalservices

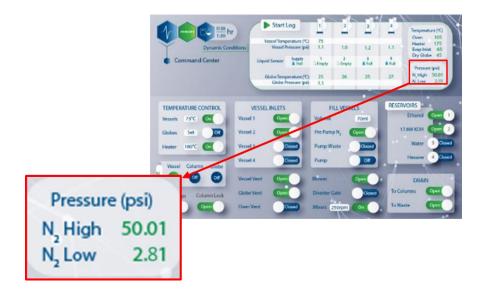




6.7 Setting Nitrogen Pressures

To confirm that the nitrogen levels are set correctly, select "DIAGNOSTICS", and then select "COMMAND CENTER." The N₂ high pressure should be between 50-51 psi and the N₂ low pressure should be 2.8 (\pm 0.2 psi).

If the N_2 high pressure or the N_2 low pressure does not fall within the acceptable ranges, follow the instructions below for "Adjusting N_2 High Pressure" or "Adjusting N_2 Low Pressure."



6.7.1. Adjusting N2 High Pressure

To increase:

- a. Pull down the gray dial on the pressure regulator to unlock.
- b. Rotate dial to the right until the pressure increases to within the desired range on the HMI touchscreen.
- c. Push up to lock dial in place.

To decrease:

- a. Pull down the dial on the pressure regulator to unlock.
- b. Rotate dial to the left until the pressure decreases to within the desired range on the HMI touchscreen.
- c. Push up to lock dial in place.





6.7.2. Adjusting N₂ Low Pressure

To increase:

a. Rotate the lock disc (large disc towards the back) counterclockwise to unlock.

Lock Disc

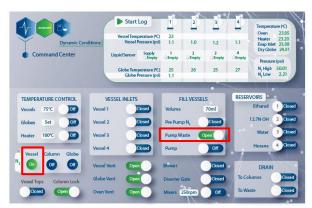
Dial

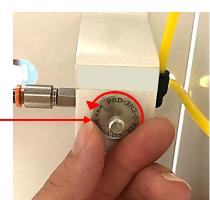
b. Rotate the dial (small knob on the front) clockwise until the pressure increases within the desired range on the HMI touchscreen.

- c. In the "Command Center", open the "Pump Waste."
- d. Turn vessel nitrogen "ON."
- e. Wait about 2 seconds.
- f. Turn Vessel N2 "Off."
- g. Close the "Pump Waste."
- h. Rotate the lock disc clockwise (to the right) to lock it in place.
- i. Confirm that the low pressure is now within range.

If N_2 Low pressure is still below 2.8 (± 0.2 psi), repeat the steps to increase.









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To decrease:

a. Rotate the lock disc (large disc towards back) counterclockwise to unlock.

Lock Disc

b. Rotate the dial (small knob on the front) one quarter turn counterclockwise.

NOTE: The user will not see a change in pressure on the HMI during this step until the residual pressure is released.

- c. In the "Command Center", open the "Pump Waste."
- d. Turn vessel nitrogen "ON."
- e. Wait about 2 seconds.
- f. Turn Vessel N2 "Off."
- g. Close the "Pump Waste."
- h. Rotate the lock disc clockwise to lock it in place.
- i. Confirm that the low pressure is now within range.

If N_2 Low pressure is still above 2.8 (± 0.2 psi), repeat steps to decrease.







6.8 Setting Nitrogen Flow Rate of Evaporation Chamber

The flow meter should only be adjusted during the initial instrument installation and does not need to be adjusted regularly. Follow the steps below to check and adjust the flow meter.

IMPORTANT: Make sure round bottom flasks are **NOT** installed in the evaporation chamber when checking the flow meter.

- 6.8.1. From the home screen, press "DIAGNOSTICS", then press "COMMAND CENTER."
- 6.8.2. Confirm that the N₂ high pressure value is within the range of 50-51 psi. If it is outside of this range, refer to (Setting Nitrogen Pressures) section.
- 6.8.3. Open the reservoir door on the instrument. The flow meter is situated within the reservoir compartment.
- 6.8.4. Turn N2 Globe "On."





- 6.8.5. The float (ball) of the flow meter should be between 7.5-8.5 sL/min.
- 6.8.6. If the reading falls outside of this range, adjust the flow meter by turning the control knob counterclockwise to increase flow or clockwise to decrease flow. This may take several rotations.
- 6.8.7. Once the float has been adjusted to be within the acceptable range, turn Globe N2 "Off."

NOTE: This step is only necessary when the instrument is first installed or if the user observes poor performance of evaporation in the round bottom flasks.





6.9 Prime Reservoir and Pump Lines

Reservoir and pump lines must be primed (i) **BEFORE the instrument is** <u>first</u> used, and (ii) when the instrument has been out of use for more than two weeks. Wear gloves and safety glasses when running the Line Prime method.

To prime all lines, complete the following steps.

6.9.1. Fill the Solution Reservoir Bottles

Fill each solution reservoir with the appropriate reagents. Reagents must match the reagents of the method that the user intends to run subsequently. Refer to Appendix A - Reagents.

You must use the correct reservoir position for each solution as some solvents must not be vented.

For further details on how to fill the reservoir bottles, refer to FLEX Service Procedure – Filling Reservoir Bottles (FLS005), located on the ANKOM website.



6.9.2. Assemble Digestion Vessels

The following parts are needed when running the line prime method: Digestion vessel glass (with or without port) and vessel bottom assembly.



6.9.2.1. Wet the bottom outside edge of the digestion vessel glass with water by wiping it with a wet paper towel. This makes for easier assembly.



6.9.2.2. Place the digestion vessel glass upside-down on the counter. Place the vessel bottom assembly on the digestion vessel glass and press down firmly until the glass seats evenly in the vessel bottom assembly.

IMPORTANT: Make sure the digestion vessel glass seats evenly and tightly, with the glass all the way down in the vessel bottom assembly, otherwise the digestion solution will leak.



6.9.3. Load Method: Line Prime

- 6.9.3.1. From the Home Screen, press "METHODS."
- 6.9.3.2. Select the "MISCELLANEOUS" category.
- 6.9.3.3. Select the "Line Prime" method.
- 6.9.3.4. The screen will display the steps required to start a run. Press the first step, "1. Enter sample name."





- 6.9.3.5. Enter "Prime" as the sample name for the first position only.
- 6.9.3.6. Press "SAVE."

NOTE: You will need to still install all four Digestion Vessels for the Line Prime Method.

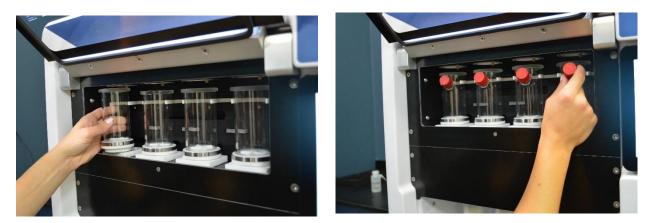
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6.9.4. Install Digestion Vessels

- 6.9.4.1. Open the digestion oven door by pulling it open.
- 6.9.4.2. Make sure the vessel tops are open. If they are not open, press the "Vessel Top" toggle so that they are in the open position.



6.9.4.3. Install the digestion vessels into the instrument by sliding them into the guide and setting them into the white vessel bases. If you are using digestion vessels with ports, make sure the ports are facing forward.



- 6.9.4.4. Close the vessel tops by pressing the "Vessel Tops" toggle. You will see the vessel tops descend and seal the digestion vessels.
- 6.9.4.5. Once all four digestion vessels have been installed, press "YES."

6.9.5. Manually Close Digestion Oven Door



6.9.6. Install SPE Columns

SPE columns must be installed for the line prime method to function correctly. They will not be wetted and can be reused. Any ANKOM SPE column can be used during this step. (e.g., FLEX-SPE-01)

To assemble fat SPE columns:

- 6.9.6.1. Remove all packaging material from inside the columns. Do not remove the white diffuser disk that holds the sorbent in place.
- 6.9.6.2. Wipe the inside of the top of the SPE column (fat column shown here) and the outside of the column funnel with a wetted paper towel (distilled water) to remove sorbent dust before pressing them together.





6.9.6.3. Gently place the column funnel inside the SPE column. **You DO NOT need to press the parts together tightly.** The instrument will apply the proper amount of force to ensure a good seal.



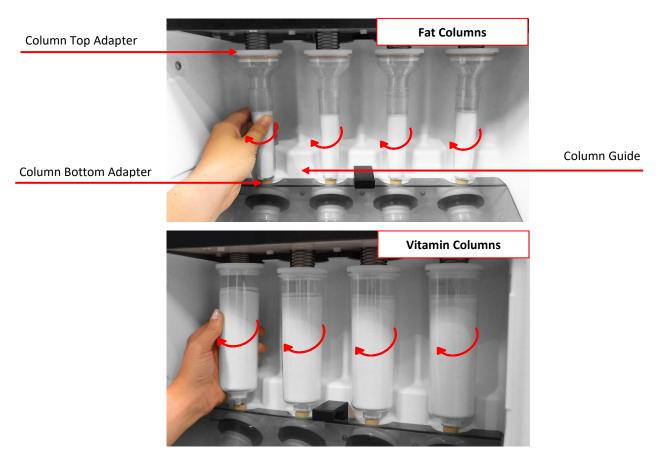
To install vitamin and fat SPE columns:

6.9.6.4. Before installing each SPE column onto the instrument, wipe the inside of the column top (vitamin) or column funnel (fat) and the column top adapter O-ring.





6.9.6.5. Install SPE columns by placing the top of the columns over the column top adapters on the instrument.Push the column upwards and guide the bottom of the column towards the instrument to seat in the column bottom adapter.<u>Twist each column a quarter turn to the left</u>.



6.9.6.6. Once all SPE columns are installed, press "YES."

6.9.7. Install Round Bottom Flasks

The round bottom flasks must be installed for the Line Prime method to function correctly. They will not be dirtied and can be reused.

- 6.9.7.1. Open the evaporation chamber door manually.
- 6.9.7.2. Turn the large black release ring at each position to the right until it fully tightens.
- 6.9.7.3. Install round bottom flasks over the round bottom flask adapters by pushing each one until it seals tightly.
- 6.9.7.4. Close the evaporation chamber door manually.



Round Bottom Flask Adapter

Release Ring

6.9.7.5. Press "YES," confirming that all four round bottom flasks are installed properly.

6.9.8. Confirm Solution Reservoirs are Full

- 6.9.8.1. Confirm that each solution reservoir is filled with the appropriate chemicals. (Refer to Appendix A Reagents)
- 6.9.8.2. Ensure that each reservoir cap is closed tightly.
- 6.9.8.3. Once all the reservoirs are full and caps securely fastened, press "YES."

6.9.9. Start Line Prime

When all set-up steps have been completed, press "START RUN."

The Line Prime method will take 7-10 minutes to complete.

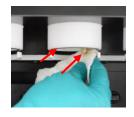


6.9.10. End of Line Prime

- 6.9.10.1. Turn the large black release ring to the left to release the round bottom flasks.
- 6.9.10.2. Remove the round bottom flasks. They should still be clean and can be used for the next run.
- 6.9.10.3. Remove digestion vessels and wash them properly before using for subsequent runs. Refer to FLEX Service Procedure End of Run Cleaning (FLS011), located on the ANKOM website.

IMPORTANT: It is very important to disassemble digestion vessels and thoroughly clean vessel bottom assemblies after use. Use water, and NOT ethanol or acetone, as a final rinse solution. When left assembled for extended periods of time (e.g. overnight), after the vessel is disassembled the O-rings in the vessel bottom assemblies will need time to decompress before reusing.

6.9.10.5. Wipe the vessel bases, the spray nozzles and O-rings in the digestion vessel tops and the column top adaptor's O-rings with a towel wetted with water.







6.9.10.6. Dispose of waste reservoir solution according to SDS guidelines and internal SOP. This reservoir will contain a mixture of all the solutions that has been used in the line prime method, which may include organic solvents, strong bases, and acids.



7. HMI Navigation

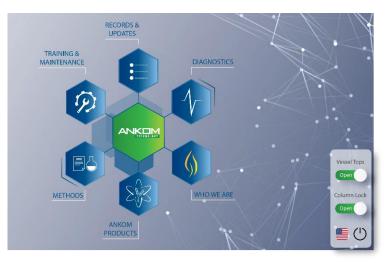
The ANKOM^{FLEX} Analyte Extractor comes equipped with revolutionary software that allows the user unlimited options for customization and method creation. The HMI has been created with the user in mind, offering preprogrammed methods, custom method capability, tracking of real-time progress, downloadable assay reports, diagnostic center accessibility, and information for troubleshooting and maintenance.

7.1 Home Screen

The home screen allows the user to see all the sections available for navigation. The user can enter a section by pressing one of the blue hexagons. To return to the home screen from any screen, the user can press the green "ANKOM" hexagon. The bar on the right side of the home screen allows the user to open and close vessel tops to gain access to the digestion vessels or to open and close the SPE column lock for access to the SPE columns. The power icon located in the lower right corner of the screen allows the user to safely shut down the instrument.

Sections on the home screen include:

- Methods
- Diagnostics
- Records & Updates
- Training & Maintenance
- ANKOM Products
- Who We Are



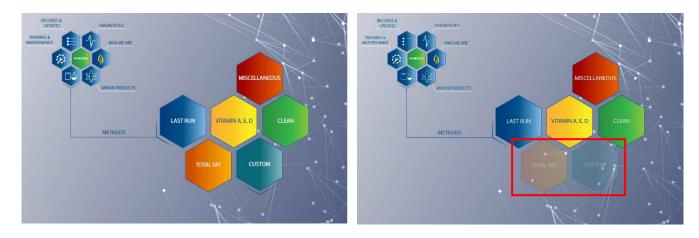
The home screen provides the user with the option to choose their preferred language.





7.2 Methods

The method section allows the user to select preprogrammed methods or to create custom methods. Faded hexagons represent methods that are available for purchase. Method availability depends on the FLEX package purchased. Method options include:



Vitamin A, E, D

This folder contains preprogrammed methods for vitamin and cholesterol analysis. For example, method "Vitamins Cholesterol" is based on a combination of official methods such as: EN-12823-1, EN-12822, and AOAC 2011.07 to recover vitamin A, E, D, and cholesterol.

Total Fat

This folder contains preprogrammed methods for total (hydrolysis) and crude fat analysis.

Miscellaneous

This folder contains other preprogrammed methods such as "Line Prime".

Custom

This option gives the user the ability to create custom methods. Custom methods are created by building sequences of modular functions, such as digestion time/temp, solution delivery, mixing, evaporation etc. This section is a valuable tool for method development and research.

Last Run

This option allows the user to select the method that was last executed. This gives easy access to methods that are run on a regular basis.

Clean

This folder contains a cleaning method used to rinse digestion vessel nozzles and vent lines.

NOTE: Method programs are available for purchase by phone or in the website product catalogue.

Part # FLEX-M-C: Enables user the ability to use custom on the instrument. This allows for the creation of new, original methods for research and development.

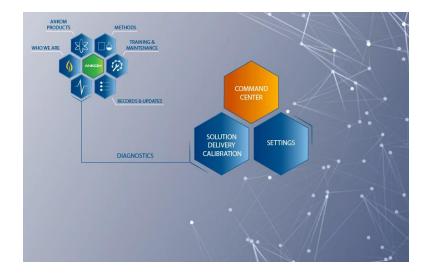
Part # FLEX-M-VC: Enables user the ability to run the Vitamins and Cholesterol method. This method is used to isolate Vitamin A,E,D and Cholesterol within a given method. This method is applicable to food and feed samples.

Part # #FLEX-M-F: Enables user the ability to run the Total Fat and Crude Fat method. These methods are applicable to oil, food, feed, and liquid samples.



7.3 Diagnostics

The diagnostics section displays the solution delivery calibration, command center, and settings options.

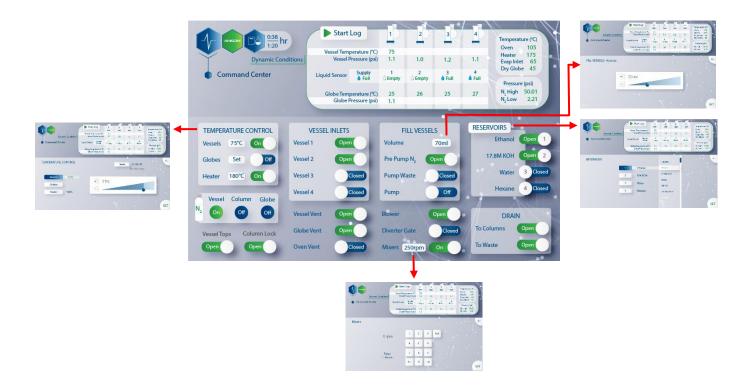


Solution Delivery Calibration

This calibration should be done when a new chemical is added or if the user would like to confirm that solution delivery is accurate. Refer to section "QC & Calibrations" for details on how to set up and run a solution delivery calibration.

Command Center

This screen contains two sets of information. The top of the screen shows the dynamic conditions of the instrument: realtime temperature and pressures. The lower half of the screen contains toggles that control the function of the instrument. These can be toggled for diagnostic purposes.





Settings

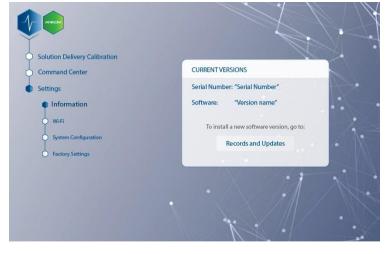
This option contains basic information, such as: serial number, software version, wi-fi networks, system configuration, and factory settings.

Information

This section displays the instrument serial number and current software version.

Wi-Fi

This section displays the networks that are available for connection. This allows remote access for troubleshooting and calibrations.



Remote Access Disclaimer:

ANKOM Technology's remote access is powered by Zoho Assist remote support. This function's purpose is to support customers from a distance through web-based, on-demand remote support sessions.

To authorize ANKOM's access to the instrument, the user connects the FLEX instrument to internet through an available network. Once connected to the network the instrument is accessible through the remote software tool Zoho Assist. Unless otherwise specified, this assumes ANKOM is authorized to unattended access to the instrument if required or necessary. ANKOM keeps all information confidential and uses this tool purely for support and diagnostics.

System Configurations

This section contains settings such as: data logging frequency, date/time, diagnostic functionality, expert mode, language options, and module and fault configurations. To make any system configuration changes, contact ANKOM for the Administrator Password.

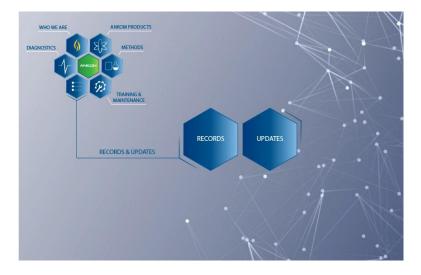
Factory Settings

This section is password protected for ANKOM's use only.



7.4 Records and Updates

The records and updates section stores reports and raw data logs and allows the user to install new software updates.



Records

This section stores various reports and logs. Reports are short summaries that are viewable on the screen and exportable via USB flash drive. Logs are raw data files that can be exported and viewed in excel or another spreadsheet program.

NOTE: To export any files from the FLEX, the USB flash drive must contain a folder labeled: ExportFiles

Assay Reports & Logs

This section contains reports and logs of all assays done on the instrument.

Calibration Reports

This section contains reports of all solution delivery calibrations done on the instrument.

Diagnostic Logs

This section contains logs that were generated through the Command Center in the Diagnostics section. Refer to section "HMI Navigation – Diagnostics".

Fault Reports

This section contains reports of all faults that occurred during a run.

Global Logs

This section contains daily logs of information such as global faults, assay start/stop times, changes in configuration settings, service events, calibration events, when updates were installed, etc.

Service Reports

This section contains reports of all services done on the instrument. This section is populated by the user or service technician.

Updates

This section allows the user to update software and methods. Refer to section "Uploading Methods and Software".

NOTE: After a software update, module and fault configuration settings will be reset to factory default settings. Although these settings have been optimized for all instruments, in rare cases ANKOM will recommend adjusting certain configuration settings that must then be reapplied after a software update. Contact ANKOM for instructions.



7.5 Training and Maintenance

This section will provide materials and information supporting training and maintenance. Materials could include video tutorials, service procedures, and analytical procedures. Files will be updated periodically. More information can always be found on the ANKOM website: www.ankom.com.



7.6 ANKOM Products

This section provides information about ANKOM Technology's products. Instruments include:

- ANKOM^{TDF} Dietary Fiber Analyzer
- ANKOM^{DELTA} Automated Detergent and Crude Fiber Analyzer
- ANKOM^{HCI} Hydrolysis System
- ANKOM^{XT15} Crude Fat Extractor
- Daisy[#] Incubator- In vitro Incubation
- ANKOM^{RF} Gas Production System Gas/Fermentation Measurement

7.7 Who we are

This section provides a look into ANKOM Technology as a company. Learn more about our American story, our foundation, and our corporate values.







8. Starting an Assay

IMPORTANT:

If the instrument has not been used in two weeks or more, wipe the O-rings and spray nozzles in the digestion vessel tops, vessel bases, and SPE column tops with a towel wetted with water. Refer to FLEX Service Procedure – End of Run Cleaning (FLS011), located on the ANKOM website.

This instrument can run saponification (e.g., vitamin and cholesterol) and acid hydrolysis (e.g., total fat) analysis. However, it is important to pay attention to the differences between these methods as they require different set-up and maintenance.

8.1 Confirm Nitrogen Pressure

Before starting an assay, make sure the $N_{\rm 2}$ High and $N_{\rm 2}$ Low pressure sensors read within operational range.

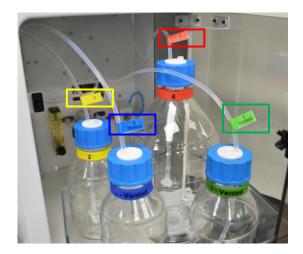
To check the pressure readings, navigate to the "Command Center" Screen: From the Home screen, press "DIAGNOSTICS", then press "COMMAND CENTER." Values should be within the following ranges:

 N_2 High pressure range: 50-51 psi N_2 Low pressure range: 2.8 (± 0.2 psi)

Item Item

8.2 Fill Solution Reservoir Bottles

Fill each solution reservoir with the appropriate reagents. Vitamin and fat analysis use different reagents. Refer to Appendix A for more details. Be sure to use the correct reservoir position (reservoir #) for each solution because certain solutions must be unvented. For further details on how to fill the reservoir bottles, refer to FLEX Service Procedure – Filling Reservoir Bottles (FLS005), located on the ANKOM website.



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8.3 Assemble Digestion Vessels

Before the user assembles the digestion vessels, make sure that each element is clean and dry. The following parts make up one fully-assembled digestion vessel: (i) Digestion vessel glass (with or without port) (PART# FLEX55 or 9362), (ii) vessel bottom assembly (PART# FLEX54), (iii) filter (PART# FLEX-VF, Vitamin and cholesterol analysis; PART# FLEX-FF, Total fat analysis; and both PART# FLEX-CF and PART# FLEX-FF, Crude fat analysis), and (iv) magnetic cross stir bar (PART# 9415). Each FLEX run needs four fully assembled digestion vessels, even if running only one to three samples.

8.3.1. Select the appropriate filter for the analysis. Place the filter (either side up) in the vessel bottom assembly. Make sure that it is pressed all the way down.

NOTE: Make sure the vitamin filter is used for vitamin and cholesterol methods, and the fat filter is used for a total and crude fat methods. If the incorrect filter is used, the filter material will degrade and plug the drainage lines and valves below the digestion vessels.

- 8.3.2. Combine the digestion vessel glass with the vessel bottom assembly. To facilitate assembly, wet the bottom outside edge of the digestion vessel glass with water by wiping it with a wetted paper towel. Do not use any other solution, as this will negatively impact the seal.
- 8.3.3. Place the digestion vessel glass upside-down on the counter. Place the vessel bottom assembly on the digestion vessel glass and press down firmly until the glass seats evenly in the vessel bottom assembly.

IMPORTANT: Make sure the digestion vessel glass seats evenly and tightly with the glass all the way down in the vessel bottom assembly, otherwise the digestion solution will leak.

8.3.4. Place the magnetic stir bar into the assembled digestion vessel.





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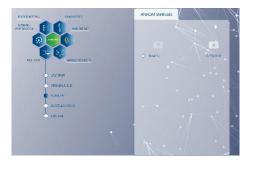


Operator's Manual

8.4 Load Method

From the Home Screen:

- 8.4.1. Press "METHODS."
- 8.4.2. Select the analytical method of choice from the pre-defined folders. Refer to the ANKOM website for the standard method procedures of each analytical method.

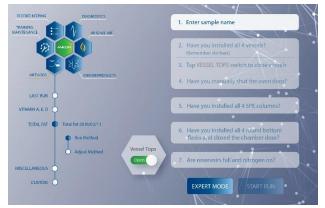


Analysis Types using the ANKOM^{FLEX} Analyte Extractor

Analysis Type	Method Description
Vitamins & Cholesterol	This method includes ethanolic saponification at 75°C for 45min, extraction through SPE (solvent: n-hexane), and evaporation of the solvent at low temperature. The analyte can then be reconstituted for chromatographic quantitation.
Total Fat	This method includes acid hydrolysis of the sample, filtration of the aqueous portion to waste, extraction of the hydrolysate with solvent, filtration with SPE, and evaporation of the solvent. The isolated fat can then be quantified gravimetrically or reconstituted for fatty acid profiling.
Crude Fat	This method includes extraction of the sample with solvent, filtration of the solvent extract through SPE, and evaporation of the solvent. The isolated fat can then be quantified gravimetrically or reconstituted for fatty acid profiling.

NOTE: Method parameters, such as solution volumes, digestion temperature and time, and mixing speed can be adjusted if needed. Please contact ANKOM for more details.

8.4.3. The screen will display the steps required to start a run. Press on step 1 to enter sample details.





- 8.4.4. Enter sample details, starting with position one. The user can run up to four samples. A green check mark indicates positions that will be run (receive solutions, drain, etc.).
- 8.4.5. The user does not need to run all positions, but positions must be run in order. For example, the user cannot run position 1 and 4 without running positions 2 and 3 as well.

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8.4.6. Press "SAVE."

NOTE: If "SAVE" is pressed before all sample names were entered, go back to the Home Screen and repeat steps to Load Method.

8.5 Install Digestion Vessels and Weigh Sample

- 8.5.1. Manually open the digestion oven door by pulling it open.
- 8.5.2. Make sure the vessel tops are open. If they are not open, press the "Vessel Top" toggle so that they are in the open (up) position.



8.5.3. Weigh sample into digestion vessels. This step is specific to each method.

Vitamins and Cholesterol:

Generally, 0.5-10g of sample is weighed directly into the digestion vessels on a precision (two-decimal) balance.

Total and Crude Fat:

0.25-1g of sample is typically weighed into a weigh tin/boat (dry samples) on an analytical (4-decimal) balance and quantitatively transferred into the digestion vessels or weighed into the digestion vessels after the vessels had been installed on the instrument (oil and liquid). For more details on specific sample size and sample preparation, refer to the method SOPs on the ANKOM website.



How to weigh dry and liquid samples for vitamin and cholesterol analysis

- Weigh dry or liquid sample directly into the assembled digestion vessel. It is very important to not exceed the specified sample size. Exceeding the sample size could result in the filters plugging.
- Install the digestion vessels into the instrument by sliding them into the guide and setting them into the white vessel bases of the oven. If you are using digestion vessels with ports, make sure the ports are facing forward and the red caps are screwed on tightly.



How to weigh oil samples for vitamin and cholesterol analysis

- Vitamin filters are hydrophobic, therefore hydrophobic samples such as oils will seep into and leak through the filter. To weigh oil sample into the digestion vessel:
 - Tare weight of assembled digestion vessel
 - Add 9.5g water into the digestion vessel
 - With a pipette, add 0.5g oil sample into the digestion vessel

It is important that the total weight of the sample weight and water equals 10g +/- 0.5g.

- Install the digestion vessels into the instrument by sliding them into the guide and setting them into the white vessel bases of the oven. If you are using digestion vessels with ports, make sure the ports are facing forward and the red caps are screwed on tightly.
- Once all four digestion vessels have received sample, press "YES."
- Press "Vessel Tops" toggle to close vessel tops. Be sure to not accidentally open the vessel tops if oil samples are contained in the digestion vessels.

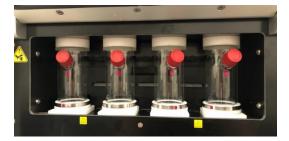
Operator's Manual

How to weigh dry sample for fat analysis

- Tare the weight of an empty weigh tin/boat on an analytical balance.
- Weigh sample into the weigh tin/boat
- Quantitatively transfer the sample into the assembled digestion vessels. If necessary, use a static free brush to make sure all the sample is transferred.
- Install the digestion vessels into the instrument by sliding them into the guide and setting them into the white vessel bases of the oven. If you are using digestion vessels with ports, make sure the ports are facing forward and the red caps are screwed on tightly.

How to weigh liquid or oil sample for fat analysis

- Install the digestion vessels into the instrument by sliding them into the guide and setting them into the white vessel bases of the oven. If you are using digestion vessels with ports, make sure the ports are facing forward and the red caps are screwed on tightly.
- Close the vessel tops.
- For oil samples only, first pipette 5ml of distilled water through the ports. This is not necessary for other liquid samples.
- Pipette sample: Using a disposable pipette, draw sample into the pipette.
- Weigh the pipette with the liquid sample.
- Insert the liquid sample through the digestion vessel port.
- Reweigh the pipette and subtract the initial weight from the final weight to calculate actual sample weight.
- Close the digestion vessel ports with the red caps.













8.6 Manually Close Digestion Oven Door

After manually closing the digestion oven door, press "Yes" on the screen.

8.7 Install the SPE Columns

The vitamin and cholesterol SPE columns (part# FLEX-SPE-01) can be installed as is onto the instrument, after the shipping packaging is removed. **Do not remove the white diffuser disk**. SPE columns are a one-time use and must be disposed of after an assay.

The total and crude fat SPE columns (part# FLEX-SPE-02) must be assembled before installed onto the FLEX instrument. The column funnel (part# FLEX9001) is reusable. ANKOM recommends replacing after approximately 20 runs. The fat SPE columns are a one-time use and must be disposed of after an assay.

- 8.7.1. Assemble Fat SPE Columns
- Remove all packaging material from inside the columns. Do not remove the white diffuser disk.
- Wipe the inside of the top of the SPE column and the outside of the column funnel with a paper towel wetted with water to remove sorbent dust, before pressing them together.
- Gently place the column funnel inside the SPE column. You DO NOT need to press the parts together tightly. The instrument will apply the proper amount of force to ensure a good seal.







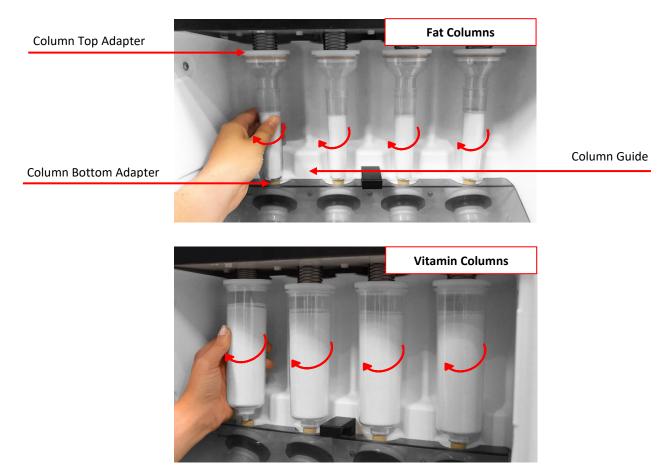
8.7.2. Install SPE Columns

• Before installing each SPE column onto the instrument, wipe the inside of the column top (vitamin) or column funnel (fat) and the column top adapter O-ring.





• Install SPE columns onto the instrument by placing the top of the columns over the column top adapters. Push the column upwards and guide the bottom of the column toward the instrument to seat in the column bottom adapter. <u>Twist each column a quarter turn to the left to ensure a good seal</u>.



8.7.3. Once all SPE columns are installed, press "YES."



8.8 Install Round Bottom Flasks

- 8.8.1. Open the evaporation chamber door manually.
- 8.8.2. Turn the large black release ring to the right until it tightens. The release ring now is out of the way, ready to receive the round bottom flask.
- 8.8.3. Install round bottom flasks over the round bottom flask adapters in the evaporation chamber by pushing each until it seals tightly.
- 8.8.4. Close the evaporation chamber door manually.
- 8.8.5. Press "YES," confirming that all four round bottom flasks are installed properly.



Round Bottom Flask Adapter

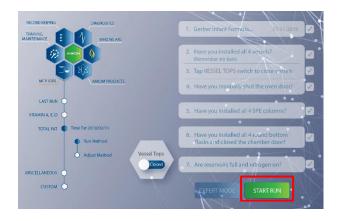
Release Ring

8.9 Confirm Solution Reservoirs are Full

- 8.9.1. Confirm that each solution reservoir is filled with the appropriate chemicals. (Refer to Appendix A Reagents)
- 8.9.2. Ensure that each reservoir cap is closed tightly.
- 8.9.3. Once all reservoirs are full, press "YES."

8.10 Start Run

When all set-up steps have been completed, press "START RUN."





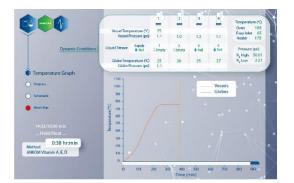
8.11 Track Progress

During an assay, the user can track progress and observe dynamic conditions. Once the run has started, the user can toggle between the following three screens to see details such as: which vessels are being filled, whether draining is complete, which reservoir is engaged, and the current step of the method.

Schematic



Temperature Graph



8.12 Abort Run

If the user no longer wants to continue with an assay, press: "Abort Run."

After pressing "Abort run", a screen prompt will ask, "Do you want to drain the content of the vessels to waste?"

- If the user does not want to keep the content in the vessels, they will select "Drain."
- If the user wants to keep the content in the vessels (to restart an assay), they will select "Do NOT Drain".



After a run is aborted, the digestion oven will cool to a safe temperature. Be careful when removing the digestion vessels because they may still contain liquid. Digestion vessels have drainage holes that will allow liquids to freely drain, once removed from the instrument.

Progress



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8.13 Clean Vessel Tops

When a run has ended the user has the option to clean the vessel tops and delivery lines through pre-programmed method. This is only **required** if the last run of the day was a **total fat analysis**. Total fat methods use hydrochloric acid (HCl) which is a very corrosive chemical. The cleaning step post-run is not necessary for vitamin, cholesterol, and crude fat methods.

NOTE: Before running this process, have the Rubber Cleaning Strip (part# 9479) on hand. It is provided with the instrument.

8.13.1. The following screen is displayed when a run has ended. Select one of the two options below:

"FINISH"- This will skip the clean step, complete the run, and take the user back to the homepage. Please continue to Step 8.14 (End of Run).

"CLEAN"- This will begin the process of cleaning the vessel tops and delivery lines. This is required if the last run of the day used HCl.

8.13.2. If the user selected "CLEAN", the following screen will be displayed. Follow the steps listed on the screen.





8.13.3. Open the vessel tops and place the rubber cleaning strip over all four vessels to completely cover them. Push the rubber cleaning strip all the way to the back of the instrument. It will function with either side facing up.

Vessels must contain a fat filter to prevent particulate from obstructing the drainage lines. It is not necessary to use a clean filter for the rinse procedure.

The cleaning strip allows water to accumulate and rinse the vessel tops and spray nozzles. The four slits in the rubber strip allow water to flow into the vessels after the tops are thoroughly rinsed and vessel pressure is turned on.





- 8.13.4. Fill the water reservoir at least half full.
- 8.13.5. Close the vessel tops by toggling the Vessel Tops tab on the screen.





- 8.13.6. Close the digestion oven door.
- 8.13.7. Press "START CLEAN" to start the rinse procedure.

The button will flash during the cleaning procedure, which will take approximately 4 minutes.

The liquid will push through the rubber cleaning strip as the process nears completion.



8.13.8. When the cleaning process is complete, the user can press "FINISH" to return to the home screen.



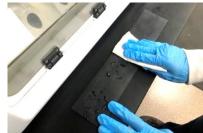


8.13.9. On the home screen toggle "Vessel tops" to open the vessel tops.



8.13.10. Remove and wipe off any residual liquid on both sides of the rubber cleaning strip.

It is recommended to wear gloves while cleaning as the user may encounter trace amounts of the corrosive chemical.



8.14 End of Run

At the end of an assay, the round bottom flasks will contain the isolated analyte. Remove the round bottom flasks for further quantitative analysis.

- 8.14.1. Turn the large black release ring to the left to release the round bottom flasks.
- 8.14.2. Remove the round bottom flasks and immediately cover each flask with a flask stopper or aluminum foil. Further quantitative analysis is described in the relevant standard operating procedures, located on the ANKOM website.
- 8.14.3. Remove and clean digestion vessel glasses for subsequent use.

IMPORTANT: It is very important to disassemble digestion vessels and thoroughly clean vessel bottom assemblies after use. Use water, and NOT ethanol or acetone, as a final rinse solution. When left assembled for extended periods of time (e.g. overnight), after the vessel is disassembled the O-rings in the vessel bottom assemblies will need time to decompress before reusing.

• Push the vessel bottom assembly off the digestion vessel glass.





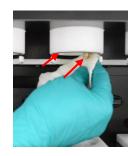
• Remove the magnetic cross stir-bar.



- With a thin tool, push the vessel filter out of the vessel base through the hole in the bottom. Discard the filter.
- Clean the digestion vessels glasses, vessel bases, and magnetic cross stir-bars for subsequent use. Use water as a final rinse solution. Do not use ethanol or acetone.



- 8.14.4. Remove and dispose of the SPE columns according to SDS guidelines and internal SOP.
- 8.14.5. Wipe the vessel bases, the spray nozzles and O-rings of the digestion vessel tops and column top adaptors with a towel wetted with water.







8.14.6. At the end of each day dispose of the waste reservoir contents. This reservoir will contain a mixture of all the solutions that has been used in the day's assays, which may include organic solvents, strong bases, and acids. Dispose according to SDS guidelines and internal SOP. Failure to do this may cause corrosion.

If the user plans to not use the FLEX instrument for longer than one week, we recommend that the instrument lines be flushed from all solutions. Refer to FLEX Service Procedure - Instrument Out of Use (FLS013), located on the ANKOM website.



8.15 Powering Down Instrument

If the user would like to turn off the FLEX, press the Power icon located in the lower right corner of the Home screen, and then press "SHUT DOWN." Once the screen is black, the user can turn off the power switch on the left side of the instrument.

NOTE: It is recommended to shut down the HMI touchscreen as described above before turning off the power switch. If the instrument needs to be restarted, wait 30 seconds before powering back on.

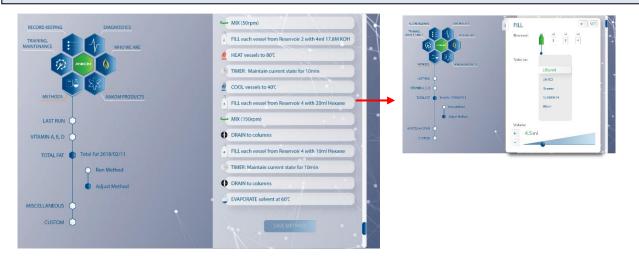




9. Adjust Method

- **9.1** From the Home Screen, select "METHODS."
- 9.2 Select the desired method category. Example: "Vitamin A, E, D."
- **9.3** Select the method of choice. A list of previously used methods will appear on the right side of the screen. Example: "Vit AED 75C 45m"
- **9.4** Select "Adjust Method." The right side of the screen will display the method sequence listing each module (step) of the method. Each of the modules (Fill, Heat, Cool, Mix, Drain, etc.) can be selected and individual parameters can be adjusted.

NOTE: The method sequence cannot be changed, and modules cannot be removed. The user must upgrade to access the custom section of the software for the ability to create a completely personalized method.



9.5 Select a module to adjust.

- FILL module- Use the slide bar to adjust the volume and select the solution that will go into the vessels.
- MIX module- To adjust the speed of the mixers, use the keypad to specify the mix speed in RPM. Select "SET" to save the parameter or the back arrow to exit without saving changes.
- HEAT vessels module- To adjust the temperature of a digestion, use the slider bar or the plus and minus buttons. To adjust the duration, tap the current digestion time and use the keypad to specify a new digestion time. Select "SET" to save the parameters or the back arrow to exit without saving changes.
- DRAIN to column module- To adjust drain to column, tap the column or waste icons to specify drain direction. Use the keypad to specify maximum drain time. Select "SET" to save the parameters or the back arrow to exit without saving changes.
- COOL vessels module- To adjust oven cool temperature, use the slider bar or the plus and minus buttons. Select "SET" to save the parameter or the back arrow to exit without saving changes.
- TIMER module- Use the keypad to specify timer. Select "SET" to save the parameters or the back arrow to exit without saving changes.



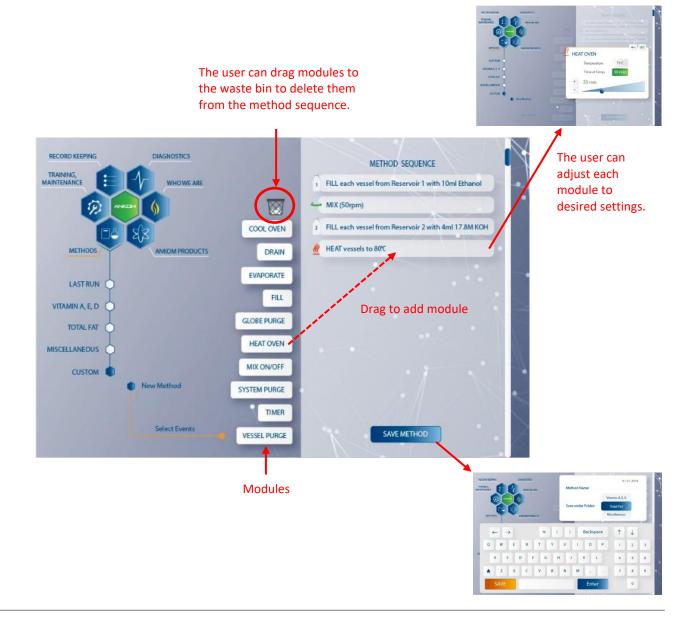
10. Custom Method

This section is only available to users who have purchased an upgrade. For full details on how to create a method refer to the ANKOM website. Custom functionality allows the user to customize an existing method or build a new method from scratch.

10.1 From the Home Screen, select "METHODS."

10.2 Select "CUSTOM."

- 10.3 Select "New Method."
- **10.4** To build a method the user will drag a module to the right side of the screen under Method Sequence. Each module can be adjusted to the user's desired setting.
- **10.5** Press "SAVE METHOD." The user will type in a method name and select which folder to save the method under.



11. Uploading Methods or Software

This section explains how to upload methods or software onto the FLEX instrument.

- **11.1** From the Home Screen, press the "Records & Updates" hexagon.
- 11.2 Press the "Updates" hexagon.
- 11.3 Select "Update Methods."

- **11.4** Insert the USB drive, that contains the new method, into the USB port on the side of the instrument.
- **11.5** The following screen will display to ask you to confirm whether you would like to proceed. If yes, press "Continue."

- **11.6** Once the upload is complete, the screen will prompt you to restart. Press "Restart." The screen will go black and then begin to reboot bringing you back to the home screen. This may take several minutes.
- **11.7** Remove the USB drive from the USB port on the side of the instrument.







12. Faults

When the instrument is not operating correctly, a fault message will alert the user. Faults can occur anytime whether an assay is in progress or not. If a fault occurs outside of an assay, an alert will pop-up on the screen. Refer to the Technical FAQs section on ankom.com or call ANKOM Technology.

If a fault is triggered inside of a method, there can be multiple different outcomes:

- The user is alerted, and the method will continue with the assay. No user action required.
- The user is alerted, and user action is required. Follow screen prompts to troubleshoot and press "CONTINUE." If the fault occurs again, refer to the Technical FAQs section on www.ankom.com or call ANKOM Technology.
- The user is alerted, and the instrument automatically aborts the run for safety reasons. The user will be asked whether they want to drain the content of the vessels to waste or not. Refer to the Technical FAQs section on www.ankom.com or call ANKOM Technology (315) 986-8090.



13. Periodic Maintenance

13.1 Solution Reservoirs and Filters

On a monthly basis (or more frequently depending on usage), follow the steps below to inspect and clean the solution reservoir bottles and filters.

- 13.1.1. Inspect the bottles, filters, and internal tubing, for any precipitant, particles, or foreign matter.
- 13.1.2. Clean filters by rinsing them under fast running water. Replace as needed.
- 13.1.3. Wash and rinse the bottles with water and let air dry, as needed.

13.2 Spray Nozzles in the Digestion Oven

Follow the steps below to inspect and clean the spray nozzles.

- 13.2.1. Periodically observe the spray capability during the addition of solution in the digestion vessels.
- 13.2.2. Clean spray nozzles by wiping each nozzle with a wetted cloth or paper towel after ever run.

13.3 Instrument Surfaces

Wipe down the surface of the instrument whenever spills have occurred.

13.4 Maintenance Schedule for Parts.

For purchasing, all parts are available individually or parts with an asterisk (*) are available in the FLEX Maintenance Kit (part #FLEX66) at <u>www.ankom.com</u>.

Service Interval	Part #	Description	Reference / Location	Quantity	Service Procedure
Clean with each use and replace every 125 runs	9375	Vessel Glass Port Cap	Remove from FLEX55 Digestion Vessel Glass and clean part with soap and water. Examine for any cracks and deterioration in seal.	1 per Vessel	N/A
Clean with each use and replace every	9423*	Vessel Bottom O-ring, small	Located in FLEX54 Vessel Bottom Assembly . Wipe clean with moist paper towel.	1 per Vessel	FLS039
250 runs	9424*	Vessel Bottom O-ring, large		1 per Vessel	
			Located in FLEX30 Slide Valve Assembly	8	FLS014
Clean every			behind front access panel on instrument.	4 (Rev A)	and FLS018
125 runs and replace only as needed	9147	Valve O-ring	Located in FLEX32 Vent Valve Assembly on the upper backside of the instrument	8 0 (Rev A)	FLS022 and FLS028



Clean every 125 runs and	0148	Dual Seal - Slide Bar	Located in FLEX30 Slide Valve Assembly behind front access panel on instrument.	0 4 (Rev A)	FLS014 and FLS018
replace only as needed	9148		Located in FLEX32 Vent Valve Assembly on the upper backside of the instrument	0	FLS022 and FLS028
				4 (Rev A)	
Replace FLEX71 every 250		Check Valve Assembly	Located in the back of the instrument on top of the electrical cabinet .	1	FLS023
runs	9412*	Round Belt 1/8in x 9.5in	Located below the Digestion Oven behind the front access panel.	4	FLS030
	8202*	Container Filter	Located inside the Reservoir Bottles	4	FLS035
	9231*	Vessel Top O-ring	Located in Vessel top inside the Digestion Oven. Wipe clean with moist paper towel.	4	FLS038
Clean and	9280*	Column Top O-ring	Located in the Column Top Assembly.	4	FLS034
replace only as needed	9313*	Tube- Pinch Valve	Located Inside Cabinet Left Side Panel.	1	FLS033
	9315	Spray Nozzle - Vessel	Located in Vessel top inside the Digestion Oven. Wipe clean with moist paper towel.	4	FLS031
9316		Spray Nozzle – N2	Located inside Evaporation Chamber. Wipe clean with moist paper towel.	4	FLS032

14. Troubleshooting

The ANKOM Technology website has the most current frequently asked questions (FAQs) and troubleshooting information. Visit our website to view FAQs or contact us via the links below.

For Technical support: www.ankom.com/contact/technical-services For Analytical support: www.ankom.com/contact/analytical-services



15. QC and Calibrations

15.1 Solution Delivery Calibration

The purpose of the solution delivery calibration is to confirm that the instrument pump is delivering the correct amount of solution. This calibration should be done when a new chemical is added, or if the user would like to confirm that solution delivery is accurate. Wear gloves and safety glasses when running this calibration.

15.1.1. Fill each solution reservoir with the appropriate reagents, at least half full, then close reservoir caps. Refer to Appendix A - Reagents. Be sure to use the correct reservoir position for each solution as certain solutions must be unvented (2 and 4). For further details on how to fill the reservoir bottles, refer to FLEX Service Procedure – Filling Reservoir Bottles (FLS005), located on the ANKOM website.



- 15.1.2. From the Home Screen, press "DIAGNOSTICS."
- 15.1.3. Select "SOLUTION DELIVERY CALIBRATION." The following screen will be displayed. Follow the instructions on the screen.
- 15.1.4. If calibrating for one new chemical, follow the calibration steps for that specific reservoir only. If calibrating all four reservoirs, follow the specific sequence listed below.

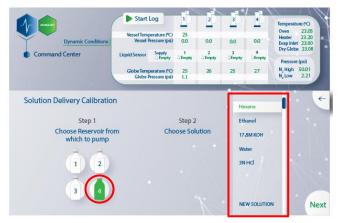
Total Fat Sequence:

- 1. Reservoir 2 3N HCl
- 2. Reservoir 1 EtOH
- 3. Reservoir 3 H₂O
- 4. Reservoir 4 Hexane

Vitamin/Cholesterol Sequence:

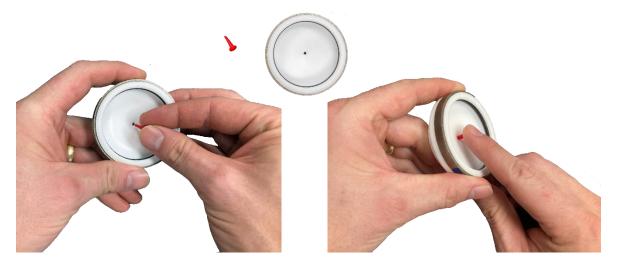
- 1. Reservoir 1 EtOH
- 2. Reservoir 2 KOH
- 3. Reservoir 3 H2O
- 4. Reservoir 4 Hexane
- 5. Reservoir 3 H2O (Run this solution again to clear lines of KOH)
- 15.1.5. A list of solutions is provided on the right side of the screen. The user will choose the solution that is in the selected reservoir and then press "Next."

NOTE: The user can add a new solution by pressing "NEW SOLUTION". The user must enter an accurate boiling point. This is used to ensure safe operating conditions. The instrument cannot run Diethyl Ether or Acetone.

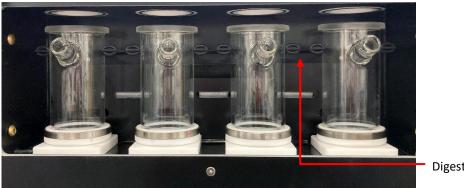




15.1.6. Insert the Vessel Bottom Plug (#FLEX72) into the Digestion Vessel Bottom. Press the plug in until it is tight and secure. This will ensure that the cap will not drain fluids.

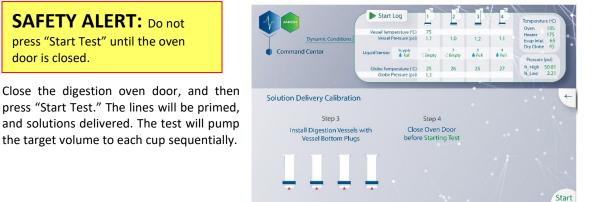


15.1.7. Install Digestion Vessels into the Digestion Vessel Guide, ensuring that the pour spout is facing towards the user. Note: The red Digestion Vessel Port Caps are not needed for this procedure.



Digestion Vessel Guide

15.1.8.



Test



Two different faults may occur during the solution delivery calibration:

Empty Supply Line

This fault is most often caused by an empty reservoir. The user must refill the reservoir and press continue.

If the message continues to be displayed, it may be caused by a restriction in the line or a leak in the system. Contact ANKOM Technology for assistance.

	Start Log	1	2	<u> </u>	4	Temperature (* Oven 10
Dynamic Conditions	Vessel Temperature (*C) Vessel Pressure (ps)	75 1.1	1.0	1.2	1.1	Heater 17 EvapInlet 6
Command Center	Liquid Serve Supply	1 Streptr	2 Ctrrety	41.e	6.0A	Dry Globe 4
	Globe Temperature (*C) Globe Pressure (pc)	25 1.1	26	25	27	N, High 50.0 N, Low 2.3
Solution Delivery Calibratio						
Emp	ty Supply Line (L5)					
The supply line sensor dia may be due to an empty					CONTIN	UE
			NUTINIE		END TE	
Please make sure that all to reprime the line or pre					END IE	ST

Full Supply Line

This message can occur if the delivery lines are not cleared properly at the end of the solution delivery calibration. Possible causes include left over liquid in the line, full waste container, or mechanical failure. Contact ANKOM Technology.

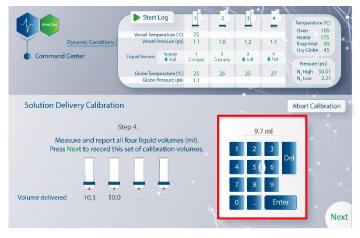
15.1.9. Open the digestion oven door and remove the digestion vessels. Using a graduated cylinder, measure and enter the volumes delivered in each Digestion Vessel to the nearest tenth of a ml (one decimal), on the HMI screen. Once the volumes have been recorded, press "Next."

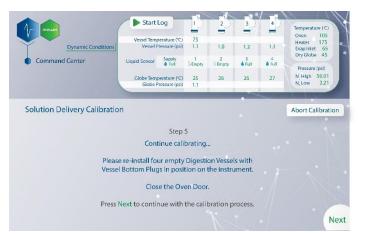


15.1.10. The following screen will be displayed when one calibration series has been completed, but calibration accuracy has not yet been reached.

> Continue with the calibration procedure by following the steps on the screen. The calibration process must be repeated until the delivery volume is within an acceptable accuracy range and the calibration test has passed.

> The system will try multiple times to achieve desired accuracy but will fault if the system reaches the allowed number of calibration attempts.







15.1.11. This screen will be displayed when the calibration is successful. The pump configuration settings for that solution are automatically updated and will be used during analytical analysis. Press "OK".

Dynamic Conditions		perature (°C) Pressure (psi)	1 75 1.1	2	3	4	Temperature (°C) Oven 105 Heater 175 Evap Inlet 65 Dry Globe 45
Command Center	Liquid Sensor	Supply Full	0 Empty	2 G Empty	3 Full	4 ♦ Full	
	Globe Tem Globe I	perature (°C) Pressure (psi)	25 1.1	26	25	27	Pressure (psi) N ₂ High 50.01 N ₂ Low 2.21
Solution Delivery Calibratio	n	_					
Calibra	ation was S	iuccessf	ul				

15.1.12. Remove the Vessel Bottom Plug (#FLEX72) from the Vessel Bottom Assembly and dispose of the plug. Vessel Bottom Plugs are intended for single use.





16. Appendix A – Reagents

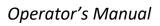
16.1 Best Practices for Solution Preparation: Total and Crude Fat Analysis

Solution	Reservoir	Solution Content	Make up
Ethanol	1. Green	Ethanol	Absolute Ethanol
3N HCI	2. Yellow		280 ml concentrated HCl (36-38%) and 720 ml DI water. Confirm density with Hydrometer (1.055-1.060 g/ml) or titration.
Water	3. Blue	Water	Deionized water
Hexane	4. Red	Hexane	ACS Grade (95%) Hexane

16.2 Best Practices for Solution Preparation: Vitamin & Cholesterol Analysis

Solution	Reservoir	Solution Content	Make up
*Ethanol	1. Green	2% (w/v) Pyrogallol (or equivalent) in Ethanol	Weigh 10g ± 0.1g Pyrogallol (or equivalent) into 500ml volumetric flask. Make up to mark with 95% Ethanol
12.7N KOH	2. Yellow	12.7N KOH	500g KOH + 500g DI water
Water	3. Blue	Water	Deionized water
Hexane	4. Red	0.05g/L BHT in Hexane	0.05g Butylated hydroxytoluene (BHT) + 1L Hexane

*Make up fresh daily. Storage Requirements: Acinic glass, Vented Reservoir, Room Temperature (15-25°C)





17. Appendix B – FLEX Maintenance: Rinsing Slide Valve

This maintenance procedure is recommended when any of the following occurred during a run:

- MF7 fault (End Drain: Time-out)
- MF27 fault (Start Drain: Time-out)
- When a run is aborted amid a drain module

These three scenarios may cause analyte and/or digestion solutions to remain in the lines and slide valve, which could result in carry-over if not properly cleaned. The maintenance procedure rinses all lines below the digestion vessels (in both directions – toward the waste reservoir and toward the SPE columns) and the slide valve.

Supplies:

- Wash bottle with water
- Empty wash bottle

Procedure:

17.1 Remove digestion vessels.

17.2 Rinse lines in the direction of the SPE Columns

- 17.2.1 On the touch screen, from the Home Screen, select "Diagnostics" and then "Command Center."
- 17.2.2 On the touch screen, select **Drain to Column: "Open"** to open the slide valve in the direction of the SPE columns.
- 17.2.3 Place the wash bottle nozzle in the small hole in the vessel base of position 1 and force water through the line until you notice clear water running into the SPE column under that position.
- 17.2.4 Repeat for all four vessel positions.

17.3 Purge remaining solution in the "Drain to Column" lines.

- 17.3.1 With an EMPTY wash bottle, manually force air through the four vessel bases.
- 17.3.2 Purge the residual solution by squeezing the wash bottle at least five times.
- 17.3.3 Select Drain to Column: "Closed" to close the slide valve.

17.4 Rinse lines in the direction of the waste reservoir

- 17.4.1 On the touch screen, from the Home Screen, select "Diagnostics" and then "Command Center."
- 17.4.2 Select **Drain to Waste: "Open"** to open the slide valve in the direction of the waste reservoir.
- 17.4.3 Place the wash bottle nozzle in the small hole in the vessel base of position 1 and force water through the line until you notice clear water running into the waste reservoir.
- 17.4.4 Repeat for all four vessel positions.

17.5 Purge remaining solution in the "Drain to Waste" lines.

- 17.5.1 With an EMPTY wash bottle, manually force air through the four vessel bases.
- 17.5.2 Purge the residual solution by squeezing the wash bottle at least five times.
- 17.5.3 Select **Drain to Waste: "Closed"** to close the slide valve.
- **17.6** Remove SPE columns and round bottom flasks.

17.7 Wipe digestion vessel bases and column top adapters with a wet paper towel.





Automation saves time and money

ANKOM Technology's products include:

	 TDF Dietary Fiber Analyzer Automates AOAC 991.43, 985.29, 2009.01, 2011.25, and 2017.16 (and associated AACC methods) IDF/SDF and TDF values Faster, Technician-free filtering Computer controlled operation Reduced per assay costs
ANKEM	 DELTA Automated Fiber Analyzer with Pump System Crude Fiber (AOCS Ba 6a-05), ADF, NDF determinations Automatically adds solutions and rinses Batch process - up to 24 samples at one time
	 XT15 Fat Extractor Official Method AOCS Am 5-04 Fully automatic Solvent recovery at 97% or greater Batch process - up to 15 samples at one time
	 RF Gas Production System High sensitivity pressure measurement Applications include Biomass-to-Energy analysis (e.g., Ethanol, methane, etc.), Biodegradability, Ruminant Nutrition, Yeast Activity, Beer/Wine Fermentation, Soil respiration, BOD, Human Digestion, etc. Wireless Computer control and data storage

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