



Rev 12/12/23

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Introduction

ANKOM Technology designs, manufactures, and markets instruments and support products used by analytical laboratories around the world in the food, feed, bio-energy, agricultural, and environmental industries. ANKOM Technology can provide you with products for determining or monitoring dietary fiber, detergent fiber, crude fiber, fat, digestibility, microbial fermentation (anaerobic or aerobic) and more.

Committed to Total Customer Satisfaction, ANKOM designs every product based on a thorough assessment of customer needs.

Congratulations on your purchase of the ANKOM^{HCl} Hydrolysis System. We are confident that this product will effectively serve your needs.

By carefully following the operating instructions in this manual, you will minimize errors in results. Experience indicates that errors in results are usually associated with minor variations in carrying out the procedure. This manual will provide you with details that will help ensure accuracy of your results.

IMPORTANT: The ANKOM^{HCl} and XT Extractors are coated with acid resistant polymer to maintain the appearance of the system. Allowing these systems to be subjected to acid contact for long periods of time will affect their appearance and should be avoided. Regular attention to periodic maintenance and proper set-up will ensure long life.

Please review the entire contents of this manual before you begin operating this product.

Warranty

ANKOM Technology warrants the ANKOM^{HCl} Hydrolysis System against any defects due to faulty 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.

Filter Bags

For optimal function, only ANKOM Technology filter bags (part # XT4) should be used in your ANKOM^{HCl} Hydrolysis System. Filter bags can be purchased from ANKOM Technology or from your local authorized ANKOM distributor.

Operating Environment

Your ANKOM^{HCl} Hydrolysis System is designed to operate within the following environments:

- Ambient Temperature Range: 15°–35°C
- Power (domestic): 100V–120V ~ 50/60Hz 10A
- Power (international): 220V–240V ~ 50/60Hz 5A

Contact Information

ANKOM Technology is committed to your total satisfaction and therefore always available to help you get the most from your ANKOM products. We are also very interested in any comments or suggestions you may have to help us improve.

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

For Sales Support: sales@ankom.com or <https://www.ankom.com/contact-us>

For Technical Support: www.ankom.com/contact/technical-services

For Analytical Support: www.ankom.com/contact/analytical-services

Telephone: (315) 986-8090

Fax: (315) 986-8091

Instrument Description

General Description

The ANKOM^{HCl} Hydrolysis System is designed to release complexed lipids from multiple food or feed samples at the same time in an automated fashion using acid hydrolysis so that the fat from the samples can then be extracted using fat solvents.

NOTE: Fat extraction can be done using the ANKOM^{XT15} and ANKOM^{XT10} Extractors.

Below is a detailed exterior view of the ANKOM^{HCl} Hydrolysis System.



Safety Precautions



Hot Surfaces – Do not touch the Vessel Lid during operation. The surface can exceed 70°C (158°F). **Failure to observe this caution may result in injury.**

Hazardous Voltages – Do not operate the instrument with the Top Cover (electrical compartment) open. Hazardous voltages are present during operation. **Failure to observe this caution may result in electrical shock or electrocution.**

Hazardous Materials – Acid solution is corrosive. Do not allow acid solution to contact the skin. Use caution when discarding waste. Dispose of waste in accordance with safe practices and regulatory provisions.

WARNING: Attempts to override safety features or to use the instruments in a manner not specified by the manufacturer 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.

NOTE:

Please review the entire contents of this manual before you begin operating this instrument.

Instrument Installation and Setup

Site Requirements

To install and operate the ANKOM^{HCl} Hydrolysis System you will need the following:

- Adjustable wrench
- Tap water connection
- Adequate power (see “Operating Environment” section)
- Due to the corrosive nature of HCl fumes it is highly recommended this instrument be placed inside a ventilation hood.

Drying capability

It is extremely important that you completely dry your food or feed samples in an ANKOMRD Dryer or an oven resistant to HCl fumes. If samples are not dried enough, residual water and acid left in the samples will lead to inaccurate fat values. Overheating samples can lead to complexing of the fats resulting in lower values.

The ANKOMRD Dryer is specifically designed for hydrolysis samples and should be set at 110°C. This will result in the appropriate temperature of 102°C inside the bag. If you are drying samples using a laboratory oven, it is recommended that you use a mechanical convection oven set to 102°C±2°.

NOTE: If you use a dryer, remember to regularly check the air flow through the filter. For details, refer to the operator's manual for your dryer.

Instrument Installation

The ANKOM^{HCl} Hydrolysis System should be installed in a clean area free of dust and excessive moisture. The instrument's power cord must be plugged into a grounded receptacle. Ensure there are no kinks or bends in any of the tubing that is connected to the instrument.

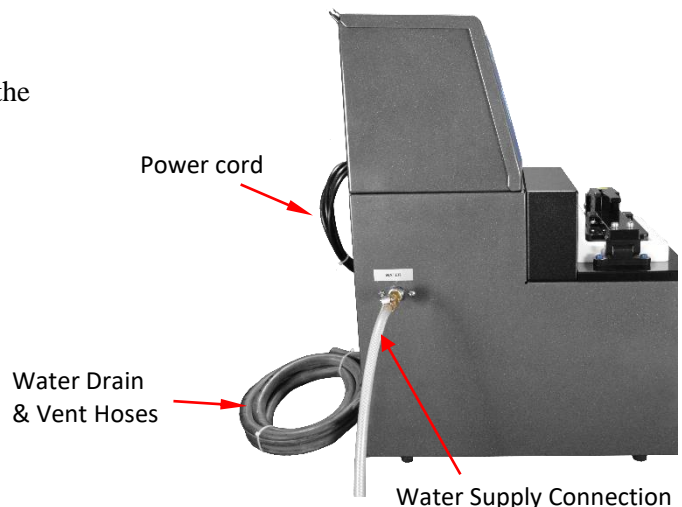
To install the ANKOM^{HCl} Hydrolysis System, follow the procedure detailed below.

1. Remove the instrument from the shipping container and set it on a smooth level surface near a drain and a cold-water supply.



WARNING: Due to the corrosive nature of hydrochloric acid, it is recommended that the instrument be operated in an acid ventilation hood separate from other instruments that are subject to corrosion.

2. Connect the instrument to a water supply using the “Water Supply Connection.”



3. Install the "Water Drain Hose" and position it to run downhill from the instrument to a drain, allowing for proper drainage and preventing condensation from accumulating inside.
4. Attach the HCl Neutralizing Filter (SKU: #H36) to the "Vent Hose" as shown below. Position the hose and the filter to run downhill from the instrument to prevent condensation from accumulating inside.



NOTE: Replace the filter every four months or when pH paper placed at the end of the filter indicates that acid fumes are exiting the filter.

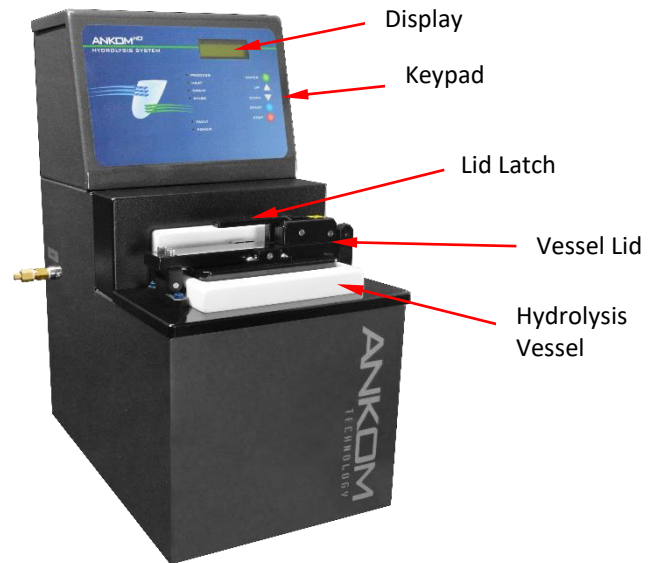
5. Plug the power cord into a grounded receptacle.

Instrument Setup

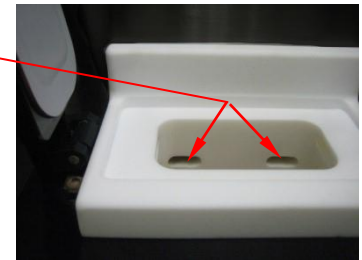
IMPORTANT: This step is extremely important to fill the waste tank in the instrument with water to dilute the waste acid from the first and subsequent runs.

To get your ANKOM^{HCl} Hydrolysis System set up and ready for use, follow the procedure detailed below.

1. Turn the instrument power switch ON. When the power is on, the switch light and the power light on the Keypad are lit.
2. Turn the water supply on.
3. Pull up on the Lid Latch to open the Hydrolysis Vessel.



4. Pour enough water into the Hydrolysis Vessel to cover the ports in the back.



5. Using the UP/DOWN arrows on the Keypad, enter an extraction time of 20 minutes.
6. Press ENTER on the Keypad.
7. Using the UP/DOWN arrows on the Keypad, enter a temperature of 90°C.
8. Press ENTER on the Keypad.
9. Using the UP/DOWN arrows on the Keypad, enter a rinse time of 10 minutes.
10. Press ENTER on the Keypad.
11. Close the Vessel Lid (a message on the Display will remind you to do this).
12. Press START on the Keypad. Within 20 minutes the water should heat to 90°C. Then the Water Drain will open.
13. Make sure that all of the water has drained out of the Hydrolysis Vessel. You should then observe two rinses with each rinse taking approximately 5 minutes to complete.

NOTE: Repeat the above procedure every three months to verify that the instrument is functioning properly.

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Hydrolysis Procedure

Total Fat Calculation

The hydrolysis procedure is done prior to fat extraction. When both hydrolysis and fat extractions are done, the total fat content can be calculated using the following formula:

$$\text{Total Fat (\%)} = \frac{(W_2 - (W_3 + (C_1 - C_2)))}{W_1} * 100$$

- Where:
- W_1 = Original sample weight (g)
 - W_2 = Bag after hydrolysis and drying (g)
 - W_3 = Bag after extraction and final drying (on ANKOM^{XT} instrument) (g)
 - C_1 = Blank bag weight, after hydrolysis and drying (g)
 - C_2 = Blank bag weight, after extraction and drying (g)

Hydrolysis Support Items

The following support items are needed to perform the hydrolysis procedure:

Item	Recommended Product
Filter Bags	ANKOM #XT4
Bag Weigh Holder (for adding sample to an empty filter bag)	ANKOM #X20
Diatomaceous Earth (DE)	ANKOM #DE1, DE2
Heat Sealer (for sealing the filter bags)	ANKOM #HS (120V), #HSi (220V)
Solvent Resistant Marker	ANKOM #F08
Multi-bag Holder (to hold bags in the Hydrolysis Chamber)	ANKOM #H33
Desiccant Pouch (for drying)	ANKOM #X45
Oven, capable of maintaining $102 \pm 2^\circ\text{C}$ (for drying)	ANKOM #RD (120V), #RDI (220V)
Electronic Balance with four-place readout	
Sample	
Spoon	

Sample Categorization

For the best results, it is important to determine the correct ratio of Diatomaceous Earth (DE) to sample. Before using your ANKOM^{HCl} Hydrolysis System for production testing, it is recommended that you categorize your samples into types and determine the standard amount of DE needed for each sample type. The following information is from testing done in the ANKOM lab. This information is meant to help you categorize your own samples.

Sample Categorization Data from ANKOM Lab *(sorted in alphabetical order by Sample Type)*

Sample Type	Fat %	DE Weight	Sample Weight	Notes
Cheddar Cheese	35%	1.2 g	0.3 g	
Cheese Curls	~ 25 %	0.9 g	0.6 g	
Chocolate Liquor	> 50 %	1.1 g	0.4 g	
Cottage Cheese	< 5 %	0.6 g	0.9 g	
Cream Cheese	15 - 35 %	1.0 g	0.5 g	
Dried Algae	15 - 20 %	0.8 g	0.7 g	
Dried Distillers Grain	< 15 %	0.5 g	1.0 g	
Dried Pet Food	5 - 15 %	0.5 g	1.0 g	
Feed Mixtures	< 10%	0.3 - 0.4 g	1.1 - 1.2 g	
Fish Feed	15%	0.75 g	0.75 g	
Forages	2 - 3 %	0.3 g	1.2 g	Most forages are light. Do not allow the mass in the bag to puff out the sides of the filter bag which will make it more difficult to completely saturate the sample.
Ground Beef	< 20 %	1.0 g	0.5 g	
Ground Beef	> 20 %	1.2 g	0.3 g	
Hot Dog	30%	1.2 g	0.3 g	
Mayonnaise	75 - 80 %	1.2 g	0.3 g	
Meat & Bone Meal	10 - 15 %	0.5 g	1.0 g	
Milk Chocolate	25 - 30 %	0.8 g	0.7 g	
Milk Powder	15 - 20 %	0.75 g	0.75 g	
Milk Replacer	> 30%	0.9 g	0.6 g	
Milk Replacer	20 - 30 %	0.75 g	0.75 g	
Moist Distillers Grain (syrup)	10 - 12 %	0.7 g	1.0 g	
Moist Pet Food (canned)	< 10 %	0.75 g	0.75 g	
Mozzarella Cheese	20%	1.0 g	0.5 g	
Potato Chips	30 - 40 %	1.2 g	0.3 g	
Powdered Non-Dairy Creamer	35%	1.0 g	0.5 g	
Pretzels	5%	0.5	1.0 g	
Processed Cheese	20 - 25 %	1.0 g	0.5 g	
Processed Cheese	> 25 %	1.2 g	0.3 g	
Sour Cream	20%	0.8 g	0.7 g	
Soybean	20%	0.75 g	0.75 g	
Swiss Cheese	30%	1.0 g	0.5 g	
Tortilla Chips	25%	0.9 g	0.6 g	
Veggie Chips	20%	0.9 g	0.6 g	
Whey Powder	2%	0.75 g	0.75 g	
Whipping Cream	35%	1.25 g	0.5 g	After adding cream to the filter bag using a pipette (see milk technique above) and not overflowing the indentation in the DE, leave the filter bag open and dry it in an oven for 1 hour at 100-105°C. Seal the bag.
Whole Milk	~ 3%	1.25 g	1.25 g	After adding the DE to the filter bag, press the eraser end of a pencil into the DE to create an indentation into which the milk can be added using a pipette. Record the weight (to make sure DE was not transferred to the pencil) & tare.
Yogurt	1 - 1.5 %	0.75 g	0.75 g	

Sample Categorization Data from ANKOM Lab (sorted by DE Weight)

Sample Type	Fat %	DE Weight	Sample Weight	Notes
Whole Milk	~ 3%	1.25 g	1.25 g	After adding the DE to the filter bag, press the eraser end of a pencil into the DE to create an indentation into which the milk can be added using a pipette. Record the weight (to make sure DE was not transferred to the pencil) & tare.
Whipping Cream	35%	1.25 g	0.5 g	After adding cream to the filter bag using a pipette (see milk technique above) and not overflowing the indentation in the DE, leave the filter bag open and dry it in an oven for 1 hour at 100-105°C. Seal the bag.
Processed Cheese	> 25 %	1.2 g	0.3 g	
Potato Chips	30 - 40 %			
Mayonnaise	75 - 80 %			
Hot Dog	30%			
Ground Beef	> 20 %			
Cheddar Cheese	35%			
Chocolate Liquor	> 50 %	1.1 g	0.4 g	
Swiss Cheese	30%	1.0 g	0.5 g	
Processed Cheese	20 - 25 %			
Powdered Non-Dairy Creamer	35%			
Mozzarella Cheese	20%			
Ground Beef	< 20 %			
Cream Cheese	15 - 35 %			
Veggie Chips	20%			
Tortilla Chips	25%			
Milk Replacer	> 30%			
Cheese Curls	~ 25 %			
Sour Cream	20%	0.8 g	0.7 g	
Milk Chocolate	25 - 30 %			
Dried Algae	15 - 20 %			
Yogurt	1 - 1.5 %	0.75 g	0.75 g	
Whey Powder	2%			
Soybean	20%			
Moist Pet Food (canned)	< 10 %			
Milk Replacer	20 - 30 %			
Milk Powder	15 - 20 %			
Fish Feed	15%			
Moist Distillers Grain (syrup)	10 - 12 %			
Cottage Cheese	< 5 %	0.6 g	0.9 g	
Meat & Bone Meal	10 - 15 %	0.5 g	1.0 g	
Dried Pet Food	5 - 15 %			
Dried Distillers Grain	< 15 %			
Pretzels	5%			
Forages	2 - 3 %			
Feed Mixtures	< 10%	0.3 - 0.4 g	1.1 - 1.2 g	

Sample Preparation

To avoid contamination of fat from one filter bag to another, it is very important to prepare your samples properly. You must first categorize your samples based on the amount of DE needed. See the “Sample Categorization” section for details.

To prepare samples for hydrolysis, follow the procedure detailed below.

1. Label/number all empty filter bags with a solvent resistant marker (SKU: #F08).
2. Place an empty filter bag in the bag holder in an open position. Record the weight of the empty filter bag (W_0).
3. Tare the weight of the empty filter bag and the holder together.
4. Add the recommended amount of DE and sample to the filter bag according to the “Sample Categorization” section.



Dry/Granular Samples

The total weight of DE and sample must NOT exceed 1.5g. If bags are overfilled, the acid will not saturate the sample during hydrolysis and artificially low recovery will be obtained.

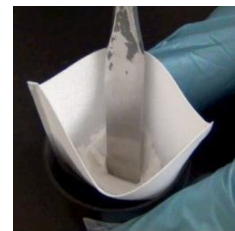
- a. Add the full recommended amount of DE to the filter bag.
- b. Record the weight of the DE and tare (W_{DE1}).
- c. Add the recommended amount of sample to the filter bag. Keep all particles away from the sealing area of the filter bag.
- d. Record the weight of the sample (W_1).



Moist Samples

The total weight of DE and sample must NOT exceed 1.5g.

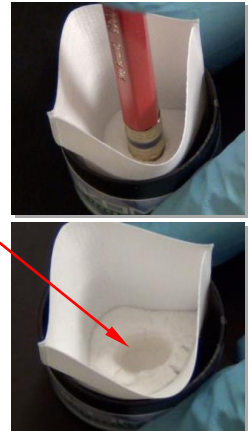
- a. Add about half of the recommended amount of DE to the filter bag.
- b. Using a spatula, make a cavity in the DE. Sample will later be weighed into this cavity.
- c. Record the weight of the DE and tare (W_{DE1}).
- d. Place the recommended amount of sample into the DE cavity, being careful that the sample does not touch the inside walls of the filter bag.
- e. Record the weight of the sample and tare (W_1).
- f. Add the other half of the DE to the filter bag to completely cover the sample.
- g. Record the weight of the second half of the DE (W_{DE2}).



Liquid Samples

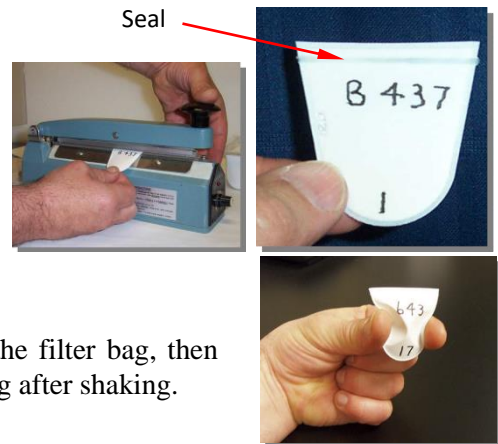
Some liquid samples with high moisture and low-fat content, such as milk, may require a total sample + DE weight of 2.5g.

- a. Add the full recommended amount of DE to the filter bag.
- b. Using a pencil eraser (or similar sized utensil), make a cavity in the DE into which the liquid sample can be later added.
- c. Record the weight of the DE and tare (W_{DE1}).
- d. Pipette the recommended amount of liquid sample into the DE cavity, being careful that the sample does not touch the inside walls of the filter bag.
- e. Record the weight of the sample (W_1).



5. Set the Heat Sealer dial to 6. (The setting may vary from sealer to sealer.)

6. Seal the filter bag within 4mm of its open end. Keep the sealer arm down for 2 – 3 seconds after the red sealer light turns off (to cool the seal). The seal can be seen as a solid melted stripe along the top edge of the filter bag (as shown to the right). If the seal is not strong, re-seal the bag.



7. For **Dry/Granular** and **Moist samples**, squeeze the edges of the filter bag, then shake it to mix or cover the sample with the DE. Flatten the bag after shaking.

8. If the sample is **liquid or clumpy** (for example ground beef) and makes the filter bag bulge, gently squeeze the bag at the bulge location to flatten it.

9. Repeat steps 3 – 9 for all filter bags that will be used in the ANKOM^{HCl} Hydrolysis System. (Up to 15 bags can be processed during one procedure.)

10. Include two blank bags (C_1 and C_2) filled with approximately 0.5g – 0.75g of DE.

IMPORTANT:

Blank bags serve as correction factors for slight losses of weight that may occur during extraction. Blank bags with DE also act as indicators of fat loss during hydrolysis. When placed next to a sample that loses fat, a blank bag will absorb some of the fat lost by the other bag. The result is a blank loss during extraction that exceeds the normal expected blank loss of 0.0025g by 0.0005g or more. This indicates that fat migrated from a bag with sample to a blank bag during hydrolysis, and then that same fat was lost during extraction. If fat migrates from the bags, a pre-extraction with solvent may be necessary.

11. Insert filter bags into the Multi-bag Holder and snap the handle in place.

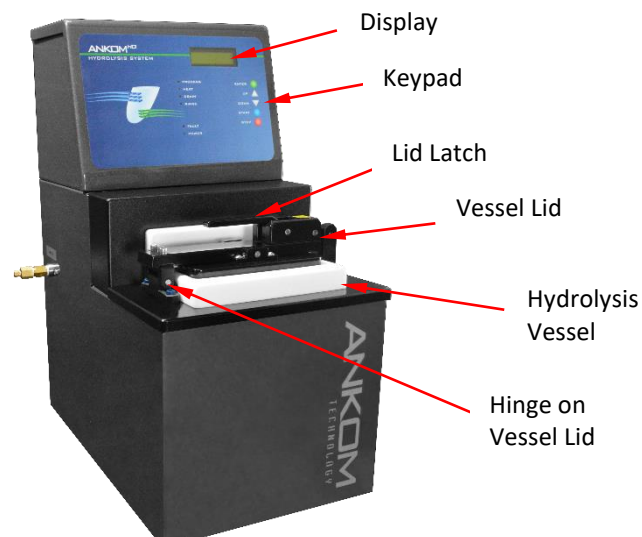
Samples are now ready for the hydrolysis procedure in the ANKOM^{HCl} Hydrolysis System.



Hydrolysis step-by-step procedure using the ANKOM^{HCl} Hydrolysis System

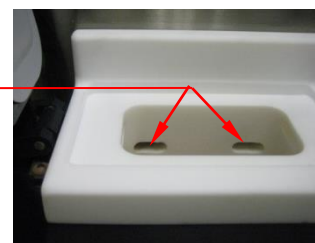
To perform hydrolysis on prepared samples, follow the procedure detailed below.

1. Turn the instrument power switch ON.
2. Turn the cold-water supply on.
3. Pull the Lid Latch up to open the Vessel Lid.
4. Place the Multi-bag Holder into the Hydrolysis Vessel.



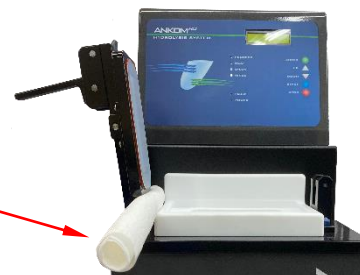
5. Pour 500ml of 3N HCl into the Hydrolysis Vessel to cover the ports in the back.

Ports in back
of Vessel



6. Roll up a piece of paper towel and place it on the Vessel Lid just above the Hinge as shown to the right. This will help prevent drips of liquid from getting on the Hinge after a run.

Rolled-up
Paper Towel



7. Push the Lid Latch down to close the Vessel Lid.
8. Press ENTER on the Keypad.
9. Use the UP/DOWN arrows on the Keypad to enter the hydrolysis time (suggested time is 60 minutes).
10. Press ENTER on the Keypad.
11. Use the UP/DOWN arrows on the Keypad to enter the hydrolysis temperature (suggested temperature is 90°C).
12. Press ENTER on the Keypad.
13. Use the UP/DOWN arrows on the Keypad to enter the rinse time (suggested time is 20 minutes).
14. Press ENTER on the Keypad.
15. Make sure the Vessel Lid is closed (a message on the Display will remind you to do this).

16. Press START on the Keypad. The ANKOM^{HCl} Hydrolysis System will automatically heat and maintain the set temperature. After hydrolysis is complete, the samples are automatically rinsed with cold water. When the instrument has completed the automated process, the Display will show "Process Complete".
17. When the automated process is complete, open the Lid Latch and immediately wipe the PTFE pad on the underside of the lid with a paper towel.

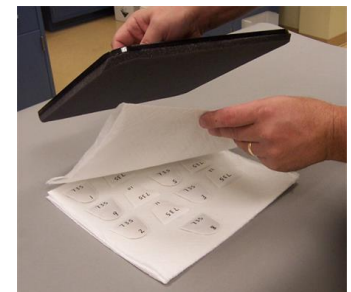
IMPORTANT This step is extremely important to prevent acid from corroding the hinge on the Vessel Lid.

18. Remove the Multi-bag Holder from the Hydrolysis Vessel.

19. Remove the individual filter bags from the Multi-bag Holder and place them on four layers of paper towel.



20. Place two layers of paper towel on top of the wet filter bags. Using the ANKOM Blotter, apply uniform pressure to the top of the paper towels for two minutes to gently press the water out of the filter bags. Blotting helps to reduce the drying time and the amount of acid residue in the bags.



21. Repeat the blotting process for two more minutes before drying.
22. Completely dry the samples in an ANKOMRD Dryer set to 110°C for three hours. This will ensure that the temperature inside the bags is at 100 - 105°C. (An oven resistant to HCl fumes can be used if it can ensure that the temperature inside the bags is at 100 - 105°C.)

IMPORTANT Residual acid left in the samples due to incomplete drying will cause corrosion in the extractor and artificially high fat values.

23. Remove samples from the dryer/oven and place them directly into a Desiccant Pouch containing pH paper.
24. Allow samples to cool to room temperature and check the pH paper. If the presence of acid shows on the paper, continue drying. If the pH paper is neutral, record the weights of the bags (W₂).

IMPORTANT If your hydrolysis results are low, it is possible that the acid was not able to completely saturate and hydrolyze the sample. In this case, it may be necessary to use no more than 1.0g of the combination of sample and DE in your filter bags. Depending on the sample content, a possible combination could be 0.5g of sample and 0.5g of DE.

The bags are now ready for extraction in the ANKOM XT Extractor.
See the specific extractor Operator's Manual for instructions.

Periodic Maintenance

Cleaning the exterior of the instrument

At the end of each day use an ammonia-based cleaner (a mild base solution) and cloth to wipe the outside of the instrument, making sure to clean the hinge on the Vessel Lid.

NOTE: Do NOT use Acetone to clean the instrument.

Cleaning the interior of the Vessel

To restore the white color to the Vessel, periodically run a bleach solution through the instrument using the procedure detailed below.

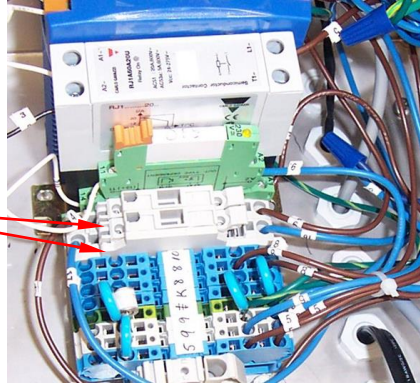
1. Turn the water supply on.
2. Pull the Lid Latch up to open the Vessel Lid.
3. Pour 600 ml or enough of a bleach solution into the Hydrolysis Vessel to cover the two ports in the back wall of the vessel.
4. Push the Lid Latch down to close the Vessel Lid.
5. Press ENTER on the Keypad.
6. Use the UP/DOWN arrows on the Keypad to enter a hydrolysis time of 60 minutes.
7. Press ENTER on the Keypad.
8. Use the UP/DOWN arrows on the Keypad to enter a hydrolysis temperature of 90°C.
9. Press ENTER on the Keypad.
10. Use the UP/DOWN arrows on the Keypad to enter a rinse time of 20 minutes.
11. Press ENTER on the Keypad.
12. Make sure the Vessel Lid is closed (a message on the Display will remind you to do this).
13. Press START on the Keypad. The bleach solution will automatically run through the instrument. When the process has completed, the Display will show "Process Complete."

Replacing the HCl Fuses

To replace the fuses in the ANKOM^{HCl} Hydrolysis System, follow the procedure detailed below.

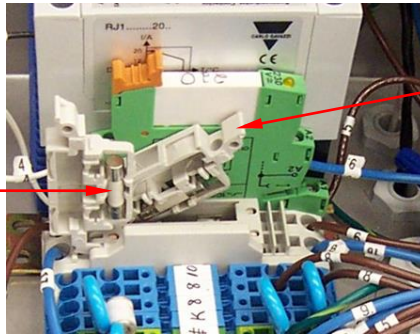
1. Turn off the instrument power and unplug the Power Cord from the outlet.
2. Remove the two screws on the Top Cover and set them aside.
3. Swing the Top Cover down exposing the electrical compartment.
4. Locate the Fuse Holders on the terminal strip (see the image on the following page). There are two fuses within the instrument; one for each pole (100 – 120V 10A or 220 – 240V 5A).

Fuse Holders



5. Press the tab and lift the Fuse Holder up.

Fuse



Tab to open Fuse Holders

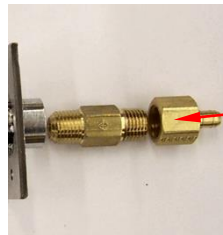
6. Replace the fuses and close the Fuse Holder completely.

7. Close the Top Cover and secure it with the two screws that you previously removed.

Cleaning/Checking the Water Filter

To clean/check the Water Filter in the ANKOM^{HCl} Hydrolysis System, follow the procedure detailed below.

1. Unscrew the Water Supply Connection and barb fitting from the HCl instrument.



Note the arrow orientation

2. Use compressed air to clean the filter.

3. Reassemble using PTFE tape on the threads.

Troubleshooting & Replacement Parts

The ANKOM Technology web site has the most current troubleshooting and replacement parts information. If you have any questions about the operation of your ANKOM^{HCl} Hydrolysis System, or if you need replacement parts, please visit www.ankom.com.

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Appendix A – Analytical Procedure

IMPORTANT: While infrequent, procedures may be updated with new information. For the most up-to-date procedure revision refer to: <https://www.ankom.com/analytical-methods-support/ankom-hydrolysis-systems>.

Total Fat by Acid Hydrolysis Filter Bag Technique using the ANKOM^{HCl} Hydrolysis System

Definition- Using Filter Bag Technology, this method determines Total Fat by releasing complexed lipids using acid hydrolysis. The fat is then capable of being extracted by fat solvents. The compounds extracted are predominantly triacylglycerides. Small amounts of other lipids having some solubility in fat solvents are also extracted.

Scope- This method is applicable to foods and feeds with 0-100% fat content.

Apparatus

1. Analytical Balance—capable of weighing 0.1 mg.
2. Dryer—forced air dryer that neutralizes acid fumes and capable of maintaining a temperature between 60-120°C (ANKOMRD Dryer, ANKOM Technology).
3. Hydrolysis instrument—capable of performing the hydrolysis at 90 ± 2°C. The instrument must also be capable of creating a uniform flow pattern around each sample to ensure uniformity of digestion (ANKOM^{HCl} Hydrolysis System, ANKOM Technology).
4. ANKOM Extractor capable of performing extractions under pressure at 90 ± 2°C (XT10, XT15, XT20, ANKOM Technology).
5. Filter Bags—constructed from chemically inert and heat resistant filter media, capable of being heat sealed closed and able to retain 1 micron particles while permitting solution penetration (XT4, ANKOM Technology).
6. Heat sealer—sufficient for sealing the Filter Bags closed to ensure complete closure (HS, ANKOM Technology).
7. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the Filter Bags (MoistureStop weigh pouch, ANKOM Technology).
8. Diatomaceous earth (DE)—(Item # DE1 or DE2, ANKOM Technology).
9. Marking pen—Solvent and acid resistant (F08, ANKOM Technology).

Reagents

1. Fat solvent used is based on the comparative method targeted. When comparing to ISO 6492 use petroleum ether (B.P. 35-65° C). When comparing to Mojonnier method use a mixture of 45% petroleum ether, 45% diethyl ether and 10% ethanol.

Caution: Most fat solvents are extremely flammable. Use a fume hood when handling exposed solvents and use the appropriate procedures to avoid static electricity discharge.

2. 3N HCl (*Note* – Some strongly bound samples will need 5N HCl)

Sample Preparation(see the *Hydrolysis Procedure* section of the *Operator's Manual* for more detail)

1. Label the filter bags using a solvent resistant marker.
2. Record the weights of all the filter bags prior to filling them.
3. Place an empty filter bag in the Bag Holder in an open position.
4. Tare the weight of the empty filter bag and the holder together.

5. Add the necessary DE and sample to the filter bag for Dry/Granular, Moist, and Liquid samples. (See the “Sample Categorization” section for help in determining how much DE to add to the filter bag.)

Note: The total weight of DE and sample **dry matter** must NOT exceed 1.5g. Too much volume inside the bags will make it difficult for the acid to completely saturate the sample during hydrolysis. This may reduce the number of bags that can be processed in the Hydrolysis Vessel at one time. For liquid samples, the total weight of DE and sample can exceed 1.5g.

Sample Preparation (continued)

Dry/Granular Samples

- a. Add the full amount of DE to the filter bag.
- b. Record the weight of the DE and tare.
- c. Add an appropriate amount of sample to the filter bag. Keep all particles away from the sealing area of the bag.
- d. Record the weight of the sample (W₁).

Moist Samples

- a. Add ½ of the amount of DE to the filter bag.
- b. Using a spatula, make a valley in the DE for the sample.
- c. Record the weight and tare.
- d. Place an appropriate amount of sample into the DE valley in the filter bag, being careful that the sample does not touch the inside walls of the filter bag so the sample can be fully covered with DE.
- e. Record the weight of the sample (W₁) and tare.
- f. Add the rest of the DE to the filter bag to completely cover the sample.
- g. Record the weight of the DE.

Liquid Samples

- a. Add the full amount of DE to the filter bag.
- b. Using a pencil eraser (or similar sized utensil), make an indentation or a space in the DE into which the liquid sample can be added using a pipette.
- c. Record the weight and tare.
- d. Pipette an appropriate amount of sample into the indentation in the DE in the filter bag.
- e. Record the weight of the sample (W₁).

6. Set the Heat Sealer dial to 6. (The setting may vary from sealer to sealer.)
7. Seal the filter bag within 4mm of its open end. Keep the sealer arm down for 2 – 3 seconds after the red sealer light turns off (to cool the seal). The seal can be seen as a solid melted stripe along the top edge of the filter bag. If the seal is not strong, re-seal the bag.
8. For Dry/Granular or Moist samples, squeeze the edges of the filter bag then shake it to mix or cover the sample with the DE. Flatten the bag after shaking.
9. If the sample is liquid or clumpy (for example ground beef) and makes the filter bag bulge, gently squeeze the bag at the bulge location to flatten it.
10. Repeat steps 3 – 9 for all filter bags that will be used in the ANKOM^{HCl} Hydrolysis System. (Up to 15 bags can be processed during one procedure.)
11. Include two blank bags (C1 and C2) filled with approximately 0.5 – 0.75g of DE.
12. Insert filter bags into the Multi-bag Holder and snap the handle in place. Samples are now ready for the hydrolysis procedure.

15. Press START on the Keypad. The ANKOM^{HCl} Hydrolysis System will automatically heat and maintain the set temperature. After hydrolysis is complete, the samples are automatically rinsed with cold water.
16. When the process is complete, the instrument will show "Process Complete" on the Display. Open the Lid Latch and immediately wipe the Teflon pad on the underside of the lid with paper towel. This will prevent moisture from corroding the Lid Latch hinge.
17. Remove the Multi-bag Holder.
18. Remove the individual filter bags from the Multi-bag Holder and place them on four layers of paper towel.
19. Place two layers of paper towel on top of the filter bags. Using the ANKOM Blotter, apply uniform pressure to the top of the paper towels for two minutes to gently press the water out of the filter bags. Blotting helps to reduce the drying time and the amount of acid residue in the bags.
20. Repeat the blotting process for two minutes before drying.
21. Completely dry the samples in the ANKOMRD Dryer or an oven resistant to HCl fumes. (ANKOM recommends drying for three hours at 100 - 105°C.) Residual acid left in the samples due to incomplete drying will cause corrosion in the extractor and artificially high fat values.
22. Remove samples from the dryer/oven and place them directly into a Desiccant Pouch containing pH paper.
23. Allow samples to cool to room temperature and check the pH paper. If the presence of acid shows on the paper, continue drying. If the pH paper is neutral, record weight (W_2) of samples.

Note: If your hydrolysis results are low, it is possible that the acid was not able to completely saturate and hydrolyze the sample. In this case, it may be necessary to use no more than 1.0g of the combination of sample and DE in your filter bags. A possible combination could be 0.5g of sample and 0.5g of DE.

24. Your samples are now ready for fat extraction in an ANKOM^{XT15} or an ANKOM^{XT10} Extractor. See the specific extractor Operator's Manual for instructions.

Note: When doing a fat extraction, use the fat solvent for the comparative method targeted. For ISO 6492, use petroleum ether (B.P. 35-65° C). For ISO 1735 (the Mojonnier method), use a mixture of 45% (v/v) petroleum ether, 45% (v/v) diethyl ether and 10% (v/v) ethanol.

Calculation (all weights in grams)

$$\% \text{ Total Fat} = \left[\frac{W_2 - (W_3 + (C_1 - C_2))}{W_1} \right] \times 100$$

Where:

- W_1 = Original sample weight (g)
- W_2 = Weight of dried sample, filter bag, and Diatomaceous Earth (DE) after hydrolysis (g)
- W_3 = Weight of dry extracted sample, filter bag, and DE (g) **(determined after using the ANKOM^{XT15} or ANKOM^{XT10} instruments)**
- C_1 = Blank filter bag dry weight after hydrolysis (g)
- C_2 = Blank filter bag weight after extraction (g)

HCl Procedure (see the Hydrolysis Procedure section of the Operator's Manual for more detail)

1. Turn the cold water supply on.
2. Using the Lid Latch, open the Vessel Lid.
3. Place the Multi-bag Holder into the Hydrolysis Vessel.
4. Pour 500ml of 3N HCl into the Hydrolysis Vessel.
5. Roll up a piece of paper towel and place it on the Vessel Lid just above the Hinge. This will help prevent drips of liquid from getting on the Hinge after a run.
6. Using the Lid Latch, close the Vessel Lid.
7. Press ENTER on the Keypad.
8. Use the UP and DOWN arrows on the Keypad to enter the hydrolysis time (suggested time is 60 minutes).
9. Press ENTER on the Keypad.
10. Use the UP and DOWN arrows on the Keypad to enter the hydrolysis temperature (suggested temperature is 90°C).
11. Press ENTER on the Keypad.
12. Use the UP and DOWN arrows on the Keypad to enter the rinse time (suggested time is 20 minutes).
13. Press ENTER on the Keypad.
14. Make sure the Vessel Lid is closed (the screen will remind you to do this).

Appendix B – Sample Categorization

Sample Categorization

For best results, it is important to determine the correct ratio of Diatomaceous Earth (DE) to sample. Before using your ANKOM^{HCI} Hydrolysis System for production testing, it is recommended that you categorize your samples into types and determine the standard amount of DE needed for each sample type. The following information is from testing done in the ANKOM lab. This information is meant to help you categorize your own samples.

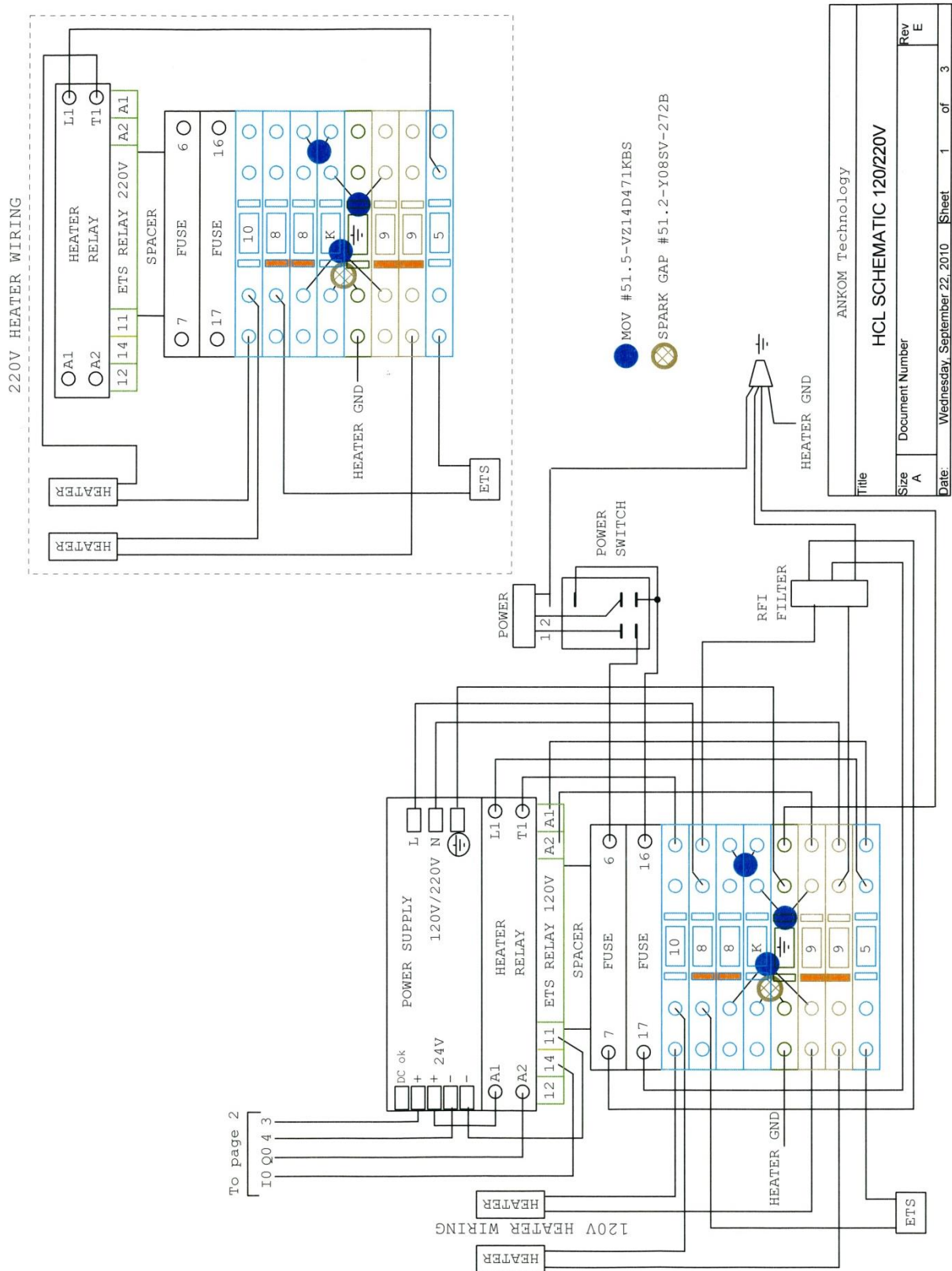
Sample Categorization Data from ANKOM Lab *(sorted in alphabetical order by Sample Type)*

Sample Type	Fat %	DE Weight	Sample Weight	Notes
Cheddar Cheese	35%	1.2 g	0.3 g	
Cheese Curls	~ 25 %	0.9 g	0.6 g	
Chocolate Liquor	> 50 %	1.1 g	0.4 g	
Cottage Cheese	< 5 %	0.6 g	0.9 g	
Cream Cheese	15 - 35 %	1.0 g	0.5 g	
Dried Algae	15 - 20 %	0.8 g	0.7 g	
Dried Distillers Grain	< 15 %	0.5 g	1.0 g	
Dried Pet Food	5 - 15 %	0.5 g	1.0 g	
Feed Mixtures	< 10%	0.3 - 0.4 g	1.1 - 1.2 g	
Fish Feed	15%	0.75 g	0.75 g	
Forages	2 - 3 %	0.3 g	1.2 g	Most forages are light. Do not allow the mass in the bag to puff out the sides of the filter bag which will make it more difficult to completely saturate the sample.
Ground Beef	< 20 %	1.0 g	0.5 g	
Ground Beef	> 20 %	1.2 g	0.3 g	
Hot Dog	30%	1.2 g	0.3 g	
Mayonnaise	75 - 80 %	1.2 g	0.3 g	
Meat & Bone Meal	10 - 15 %	0.5 g	1.0 g	
Milk Chocolate	25 - 30 %	0.8 g	0.7 g	
Milk Powder	15 - 20 %	0.75 g	0.75 g	
Milk Replacer	> 30%	0.9 g	0.6 g	
Milk Replacer	20 - 30 %	0.75 g	0.75 g	
Moist Distillers Grain (syrup)	10 - 12 %	0.7 g	1.0 g	
Moist Pet Food (canned)	< 10 %	0.75 g	0.75 g	
Mozzarella Cheese	20%	1.0 g	0.5 g	
Potato Chips	30 - 40 %	1.2 g	0.3 g	
Powdered Non-Dairy Creamer	35%	1.0 g	0.5 g	
Pretzels	5%	0.5	1.0 g	
Processed Cheese	20 - 25 %	1.0 g	0.5 g	
Processed Cheese	> 25 %	1.2 g	0.3 g	
Sour Cream	20%	0.8 g	0.7 g	
Soybean	20%	0.75 g	0.75 g	
Swiss Cheese	30%	1.0 g	0.5 g	
Tortilla Chips	25%	0.9 g	0.6 g	
Veggie Chips	20%	0.9 g	0.6 g	
Whey Powder	2%	0.75 g	0.75 g	
Whipping Cream	35%	1.25 g	0.5 g	After adding cream to the filter bag using a pipette (see milk technique above) and not overflowing the indentation in the DE, leave the filter bag open and dry it in an oven for 1 hour at 100-105°C. Seal the bag.
Whole Milk	~ 3%	1.25 g	1.25 g	After adding the DE to the filter bag, press the eraser end of a pencil into the DE to create an indentation into which the milk can be added using a pipette. Record the weight (to make sure DE was not transferred to the pencil) & tare.
Yogurt	1 - 1.5 %	0.75 g	0.75 g	

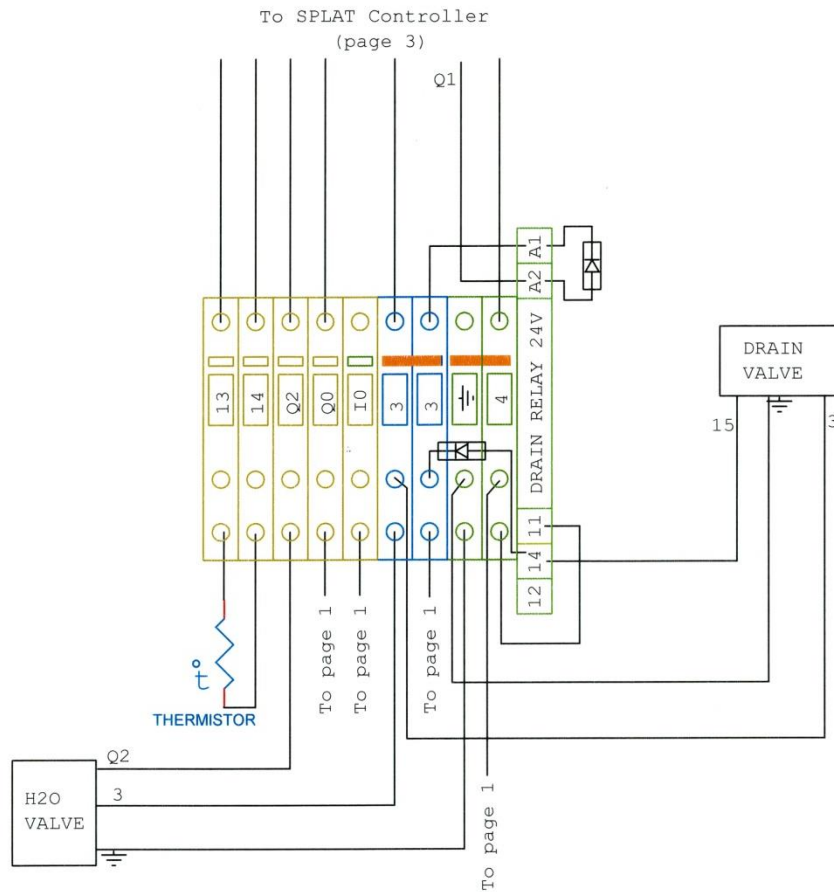
Sample Categorization Data from ANKOM Lab (sorted by DE Weight)

Sample Type	Fat %	DE Weight	Sample Weight	Notes
Whole Milk	~ 3%	1.25 g	1.25 g	After adding the DE to the filter bag, press the eraser end of a pencil into the DE to create an indentation into which the milk can be added using a pipette. Record the weight (to make sure DE was not transferred to the pencil) & tare.
Whipping Cream	35%	1.25 g	0.5 g	After adding cream to the filter bag using a pipette (see milk technique above) and not overflowing the indentation in the DE, leave the filter bag open and dry it in an oven for 1 hour at 100-105°C. Seal the bag.
Processed Cheese	> 25 %	1.2 g	0.3 g	
Potato Chips	30 - 40 %			
Mayonnaise	75 - 80 %			
Hot Dog	30%			
Ground Beef	> 20 %			
Cheddar Cheese	35%			
Chocolate Liquor	> 50 %	1.1 g	0.4 g	
Swiss Cheese	30%	1.0 g	0.5 g	
Processed Cheese	20 - 25 %			
Powdered Non-Dairy Creamer	35%			
Mozzarella Cheese	20%			
Ground Beef	< 20 %			
Cream Cheese	15 - 35 %			
Veggie Chips	20%	0.9 g	0.6 g	
Tortilla Chips	25%			
Milk Replacer	> 30%			
Cheese Curls	~ 25 %			
Sour Cream	20%	0.8 g	0.7 g	
Milk Chocolate	25 - 30 %			
Dried Algae	15 - 20 %			
Yogurt	1 - 1.5 %	0.75 g	0.75 g	
Whey Powder	2%			
Soybean	20%			
Moist Pet Food (canned)	< 10 %			
Milk Replacer	20 - 30 %			
Milk Powder	15 - 20 %			
Fish Feed	15%			
Moist Distillers Grain (syrup)	10 - 12 %			
Cottage Cheese	< 5 %	0.6 g	0.9 g	
Meat & Bone Meal	10 - 15 %	0.5 g	1.0 g	
Dried Pet Food	5 - 15 %			
Dried Distillers Grain	< 15 %			
Pretzels	5%			
Forages	2 - 3 %	0.3 g	1.2 g	Most forages are light. Do not allow the mass in the bag to puff out the sides of the filter bag which will make it more difficult to completely saturate the sample.
Feed Mixtures	< 10%	0.3 - 0.4 g	1.1 - 1.2 g	

Appendix C – Electrical Diagram (pg. 1 of 3)

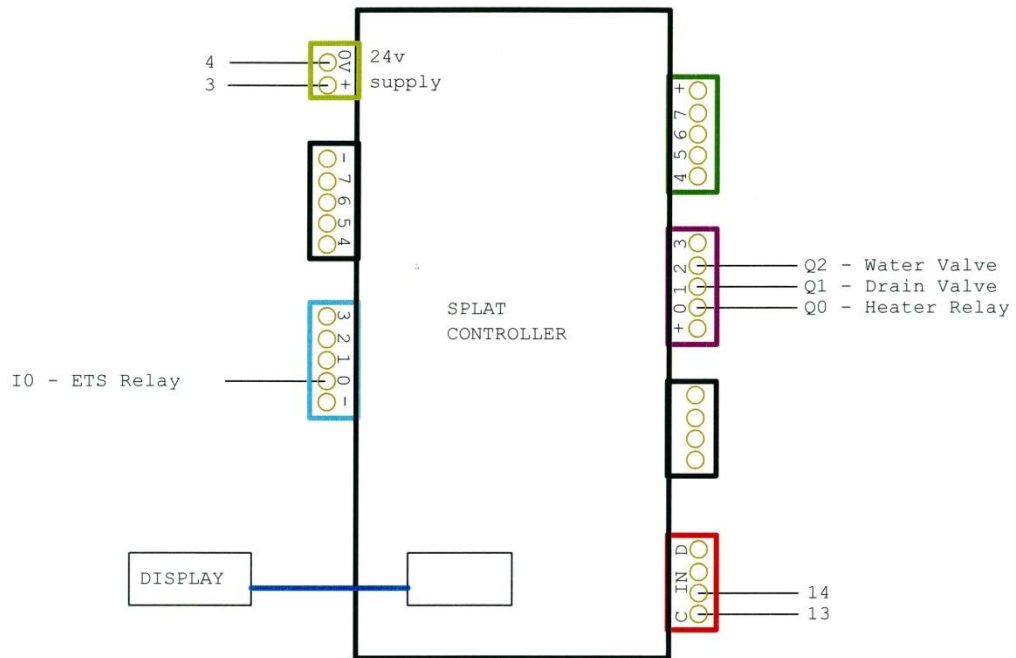


Appendix A – Electrical Diagram (pg. 2 of 3)



ANKOM Technology		
Title		
HCL SCHEMATIC 120/220V		
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		E
Date:	Wednesday, September 22, 2010	Sheet 2 of 3






Appendix A – Electrical Diagram (pg. 3 of 3)



ANKOM Technology		
Title HCL SCHEMATIC 120/220V		
Size	Document Number	Rev E
Date:	Wednesday, September 22, 2010	Sheet 3 of 3

Automation saves time and money!

ANKOM Technology is an international company with products that include...

	<p>FLEX Analyte Extractor</p> <ul style="list-style-type: none"> • Simplifies fat-soluble vitamin and cholesterol analysis • Crude and total fat analysis capability coming soon • Eliminates chemical handling to improve safety • Eliminates bi-phase extractions • Provides ability to create custom methods
	<p>TDF Dietary Fiber Analyzer</p> <ul style="list-style-type: none"> • Automates AOAC 991.43 and AACC 32-07.01 • Automates AOAC 2009.01/2011.25 and AACC 32-45.01 • IDF/SDF and TDF values • Faster, Technician-free Filtering • Computer controlled operation • Reduced per assay costs
	<p>DELTA Automated Fiber Analyzer with Pump System</p> <ul style="list-style-type: none"> • Crude Fiber (AOCS Ba 6a-05), ADF, NDF determinations • Automatically adds solutions and rinses • Batch process - up to 24 samples at one time
	<p>XT15 Fat Extractor</p> <ul style="list-style-type: none"> • Official Method AOCS Am 5-04 • Fully automatic • Solvent recovery at 97% or greater • Batch process - up to 15 samples at one time
	<p>RF Gas Production System</p> <ul style="list-style-type: none"> • High sensitivity pressure measurement • Anaerobic activity analyses (rumen, yeast, beer/wine fermentation, etc.) • Biodegradability analyses • Aerobic activity analyses (BOD, soil respiration, etc.) • Wireless Computer control and data storage

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