DAISY II INCUBATOR

Operator's Manual

Rev 12/05/24

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ANKOM TECHNOLOGY

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Introduction

ANKOM Technology designs, manufactures, and markets instruments and support products used by analytical laboratories around the world in the environmental, agricultural, biomass, and food industries. ANKOM Technology can provide you with products for determining or monitoring detergent fibers, dietary fibers, 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 Daisy^{II} Incubator. 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 assure accuracy of your results.

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

Warranty

ANKOM Technology warrants the ANKOM Daisy^{II} Incubator 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.

Filter Bags

ANKOM Technology filter bags (part #F57) are designed to support precision and accuracy in analysis. Use of other types of filtration media not tested and approved by ANKOM Technology may cause damage to electrical valves and other components and void your warranty. Filter bags can be purchased from ANKOM Technology or from your local authorized ANKOM distributor.

Operating Environment

Your ANKOM Daisy^{II} Incubator is designed to operate within the following environments:

- Ambient Temperature Range: 15−30°C (60-85°F)
- Humidity: 20–60% RH
- Power (domestic): 100 − 120V ~ 3A 60Hz
- Power (international): 220 − 240V ~ 3A 50Hz
- Bench space that can accommodate the instrument dimensions of 46.99 cm (18.5") L x 43.18 cm (17") W x 63.50 cm (25") H and a weight of 29.94 kg (66 lbs.)

Contact Information

At ANKOM Technology we are 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

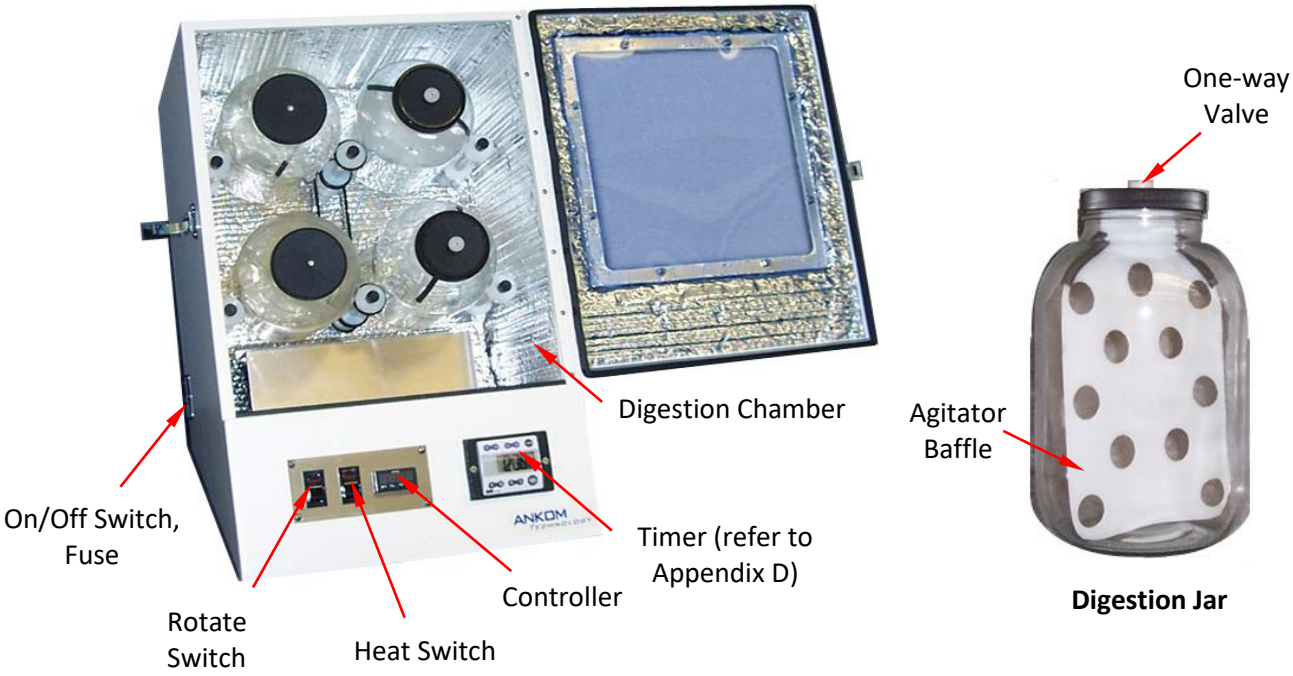
The DAISY^{II} Incubator was designed to efficiently and accurately analyze up to 100 samples individually enclosed in filter bags. You can perform either rate studies or total digestion studies in a batch operation. The DAISY^{II} instrument will free you from some of the labor requirements associated with the conventional methods. With the ANKOM system, you divide your assays between four (4) digestion jars versus a comparable conventional method, which requires 100 test tubes. Proper anaerobic conditions are easier to maintain, because the CO2 purging process is simplified. The DAISY^{II} Incubator can perform digestibility studies using enzymes or rumen inoculum. The sequential NDF assay required for In Vitro True Digestibility (IVTD) assays can easily be processed using the ANKOM$^{200/220}$ Fiber Analyzer because the filter bags are the same for both instruments. The DAISY^{II} Incubator maintains an incubation temperature of 39.5° C, while providing agitation.

If you own one of our earlier models of the DAISY^{II} Incubator you will note some slight changes in appearance. Although originally designed to provide the US research market with a low cost and efficient method for accomplishing IVTD studies, we found that the appeal for this product extended around the world. This required that we design a product that could support commercial laboratory needs. The product also needed to be able to withstand the impact of international shipments. We know that you will be pleased with these changes. Make sure that you read this manual carefully before you proceed. As a result of recent modifications and some inter-lab studies, we have made some modifications to the assay procedure. These changes provide for precise and accurate rate and digestion study results.

Please note that we also provide a full range of products to support fiber analysis and in situ studies. We offer chemicals and other ancillary products that will assist you in getting accurate results at a reasonable cost.

Below is a detailed view of the instrument.

- The **Rotate** switch controls the rotation of the digestion jars.
- The **Heat** switch allows the controller to heat the digestion jars, samples, and rumen inoculum.
- The **Controller** maintains the temperature inside the temperature at 39.5°C+/-5. Do not adjust without consulting ANKOM Technology.



Safety Precautions

Caution, Lift Hazard: Lift and move using two people only.

Caution, Rear Surface Hazardous Voltages: Do NOT operate the instrument with the back cover removed – hazardous voltages are present during operation. The power cord must be connected prior to rear panel removal. **Failure to observe this caution may result in electrical shock or electrocution.**

This system is designed to meet and/or exceed the applicable standards of CE, CSA, NRTL and OSHA.

IMPORTANT:

- All switches should be in the OFF position before plugging the power cord into the electrical outlet.
- An electrical fuse will disconnect the electrical power supply in case of malfunction.

Instrument Set-Up

Your instrument comes complete with a power cord and digestion jars. To set up your instrument, follow the steps below.

1. Set the instrument on a firm, level surface. Place the back of the instrument no closer than one inch from a wall.

   IMPORTANT: Do NOT place this instrument where it will be subject to excessive shock, vibration, dirt, moisture, oil, or other fluids.

2. Plug the power cord into the plug outlet and then into an electrical outlet.

Operation

Your Daisy^{II} Incubator is designed to provide incubation and agitation of samples at a constant 39.5°C+/-5. The controller is preset to 39.5°C. Consult ANKOM Technology if a different temperature setting is desired.

To Start the Cycle:

1. Turn the instrument's **On/Off Switch** on.
2. Add samples and solution to digestion jars as per procedure.
3. Turn the **Heat** and **Rotate** switches on. Visually confirm the jars are rotating and the heat lamps are on.

To End the Cycle:

1. Turn the **Heat** and **Rotate** switches off.
2. Empty the fluid from each jar and process the samples according to the specific procedure.

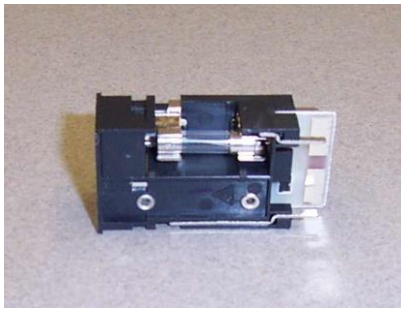
Periodic Maintenance

1. Check to make sure the **Heat** lamps are both operating.
2. To ensure jars don't slip on the drive belt, clean the outside of the jars with window cleaner.

Fuse Replacement:

Replace both fuses – 120V (3A) or 220V (3A).

Pry the fuse holder out of the On/Off switch



This is oriented for 220V operation

Troubleshooting & Replacement Parts

The ANKOM Technology web site has the most current troubleshooting and replacement parts information. Therefore, if you have any questions about the operation of your DAISY^{II} Incubator, or if you need replacement parts, please visit our web site at **www.ankom.com.**

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Appendix A – Analytical Procedures

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/daisy-incubators.

ANKOM Technology Method 3 - *In Vitro* True Digestibility using the DAISY^{II} Incubator

A. Reagents

(a) Buffer Solution A:

	g/liter
KH_2PO_4	10.0
$MgSO_4 \cdot 7H_2O$	0.5
NaCl	0.5
$CaCl_2 \cdot 2H_2O$	0.1
Urea (reagent grade)	0.5

(b) Buffer Solution B:

Na_2CO_3	15.0
$Na_2S \cdot 9H_2O$	1.0

(c) Neutral Detergent Solution

B. Apparatus

(a) DAISY^{II} Incubator
(b) Filtration device - F57 Filter Bags.
(c) Impulse bag sealer - HS/HSi Heat Sealer.
(d) Thermos
(e) ANKOM$^{200/220}$ Fiber Analyzer

C. Procedure

Preparation of Filter Bags and Sample:

Pre-rinse F57 filter bags in acetone for three to five minutes and completely air-dry. The acetone rinse removes a surfactant that inhibits microbial digestion. Weigh each F57 filter bag and record weight (W_1). Zero the balance and weigh 0.25g of sample (W_2) **directly** into filter bag. NOTE: For 48 hr studies a sample size of 0.5 g is acceptable. Heat seal bag closed and place in the **Daisy^{II} Incubator** digestion jar (up to 25 samples per jar). Samples should be evenly distributed on both sides of the digestion jar divider. Include at least one sealed blank bag for correction factor (C_1).

Preparation of (combined) Buffer Solution: *(For each digestion jar)*

a) Pre-warm at 39°C both buffer solutions (A & B). In separate container add ~266 ml of solution B to 1330 ml of solution A (1:5 ratio). The exact amount of A to B should be adjusted to obtain a final pH of 6.8 at 39°C. No further adjustment of pH is necessary. Add 1600 ml of combined A/B mixture to each digestion jar.

b) Place the digestion jars with samples and buffer solution into **DAISY^{II} Incubator** and turn on heat and agitation switches. Allow temperature of digestion jars to equilibrate for at least twenty to thirty minutes.

Preparation of Inoculum and Incubation:

Maintain all glassware at 39°C

a) Preheat two 2L thermos bottles by filling with 39° C water. Empty heated water just prior to collection of rumen inoculum. Using the appropriate collection procedure, remove at least 2000 ml of rumen inoculum and place in thermos. Include approximately two "fistfuls" of the fibrous mat from the rumen with your collection in one thermos.

b) Preheat a blender by filling with 39° C water. Empty the heated water just prior to pouring the rumen inoculum from the thermos into the blender. Purge the blender container with CO_2 gas and blend at a high speed for 30 seconds. The blending action serves to dislodge microbes that are attached to the mat and assure a representative microbial population for the *in vitro* fermentation. Filter the blended digesta through four layers of cheesecloth into a five-liter flask (pre-heated 39° C). Filter the remaining rumen fluid in the other thermos through four fresh layers of cheesecloth into the same five-liter flask. NOTE: Allow for extra cheesecloth around the edges to facilitate squeezing contents of filtered mat. The flask should be continually purged with CO_2 and continued during the transfer of the inoculum.

c) Remove one digestion jar from the **DAISY^{II} Incubator** and add the 400ml of inoculum to the buffer solution and samples. Purge the digestion jar with CO_2 gas for thirty seconds and secure lid.

d) Repeat process for all digestion jars to be used. NOTE: Do not allow CO_2 gas to bubble through the buffered inoculum, rather use the CO_2 to form a gaseous blanket over the contents of the jar.

e) Incubate for 48 hours. The **DAISY^{II} Incubator** will maintain a temperature of 39.5°C ± 0.5. If temperature of jars varies greater than one degree then move incubator to a warmer location or place blanket or similar insulator over incubator.

f) At completion of incubation, remove jars and drain fluid. Rinse bags about two times with cold tap water by pouring water into the jar, swirling, and draining. Gently squeeze excess rinse water from the bags. The purpose of the rinse is to remove the bulk of the digestive solution and water-soluble compounds without excessive shaking or aggressively squeezing of the bags.

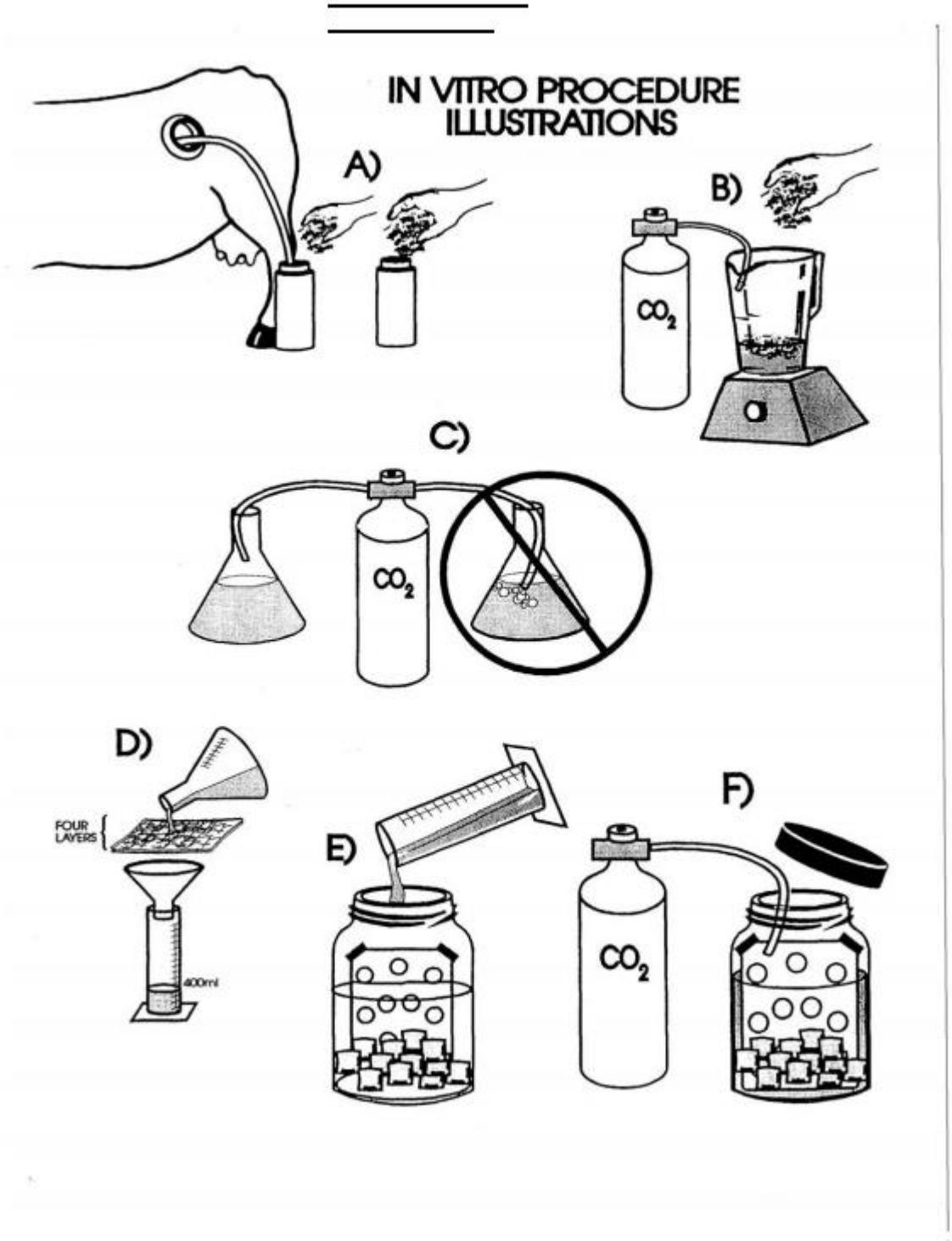
g) When determining True Digestibility, it is necessary to remove microbial debris and any remaining soluble fractions using Neutral Detergent Solution. After rinsing the bags in water place them in the **ANKOM^{200} Fiber Analyzer** and follow the procedure for determining NDF. Record the post *in vitro* NDF weight as W_3. NOTE: Bags can be stored in the refrigerator or freezer until NDF determinations can be performed.

D. Calculate:

$$\textbf{\% IVTD (as received basis)} = 100 - \frac{(W_3 - (W_1 \times C_1)) \times 100}{W_2}$$

$$\textbf{\% IVTD}_{\textbf{DM}}\textbf{ (DM basis)} = 100 - \frac{(W_3 - (W_1 \times C_1)) \times 100}{(W_2 \times DM)}$$

Where: W_1 = Bag tare weight
W_2 = Sample weight
W_3= Final bag weight after In Vitro and sequential ND treatment
C_1 = Blank bag correction (final oven-dried weight/original blank bag weight)


IN VITRO PROCEDURE
ILLUSTRATIONS
A)
B)
CO_2
C)
CO_2
D)
FOUR LAYERS
400ml
E)
F)
CO_2

Appendix B – W-1 ANKOM Technology Fiber Weighing Procedure for In Vitro Determination

Electronic Balance-

We recommend a four place readout on the balance and suggests "Balance Talk™" or other LIMS software for data input and management.

Items needed:

Electronic Balance

Heat Sealer

#HS – (120V) or #HSi- (220V)

Filter Bags

#F57

Bag Weigh Holder

#X20

Chosen Sample

MoistureStop™ Weigh Pouch

#X45

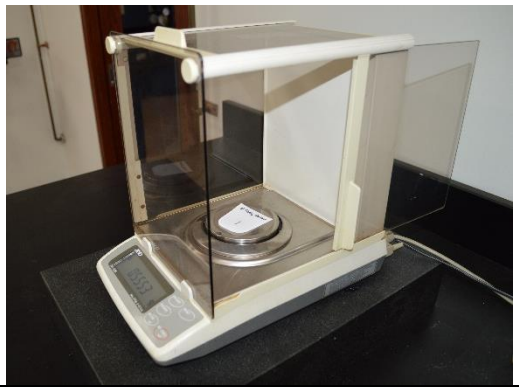
Solvent Resistant Marker

#F08

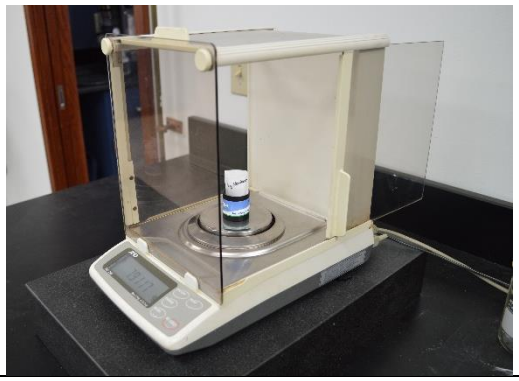
1. Wash and dry filter bags as per procedure. Number all bags using a solvent resistant marker.

2. Weigh and record filter bag weights.

*Avoid status electricity

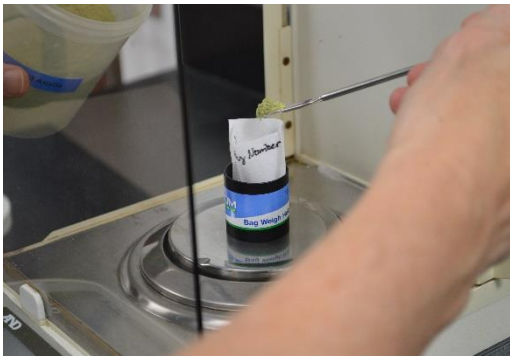


3. Tare weight of Filter Bag and Bag Weigh Holder.



4. Add 0.25g or 0.05g of sample to filter bag using a spatula. Be careful not to get sample particles on top edge of the bag sealing area.

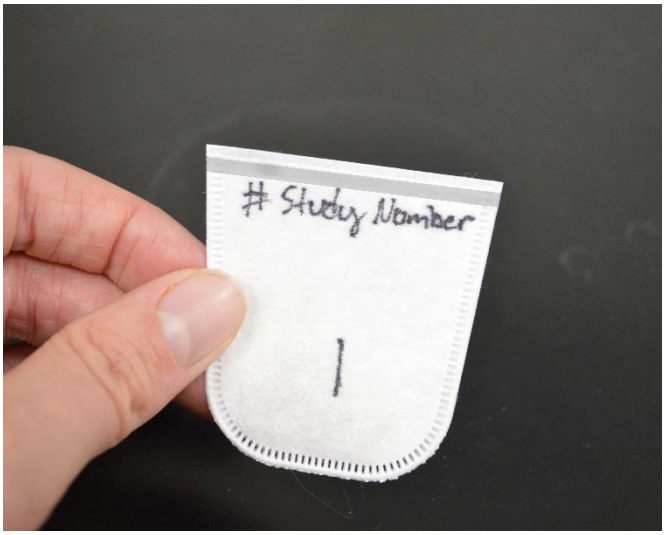
Weigh sample and record weight.



5. Heat sealer dial should be set between 4 and 5. The setting may vary from sealer to sealer.

6. Seal each Filter Bag within 4mm of its open end. Hold down handle for 2-3 seconds after the red light turns off to cool seal.

7. IMPORTANT- The seal can be seen as a solid melted stripe along the top edge. If the seal is not strong, re-seal.

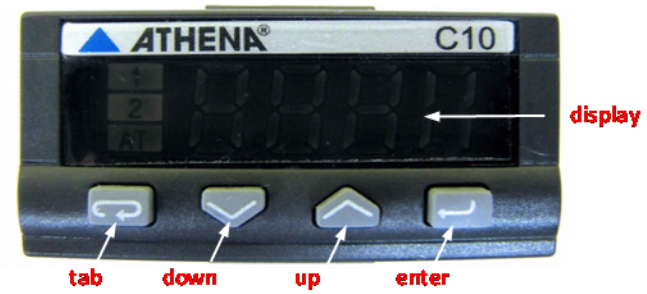


8. Perform the extraction as per procedure.

9. After digestion is complete, acetone-rinse and dry according to procedure. Remove from oven and immediately store in the MoistureStop™ Weigh Pouch.

10. Cool to room temperature and re-weigh each bag.

Appendix C – Controller Configuration



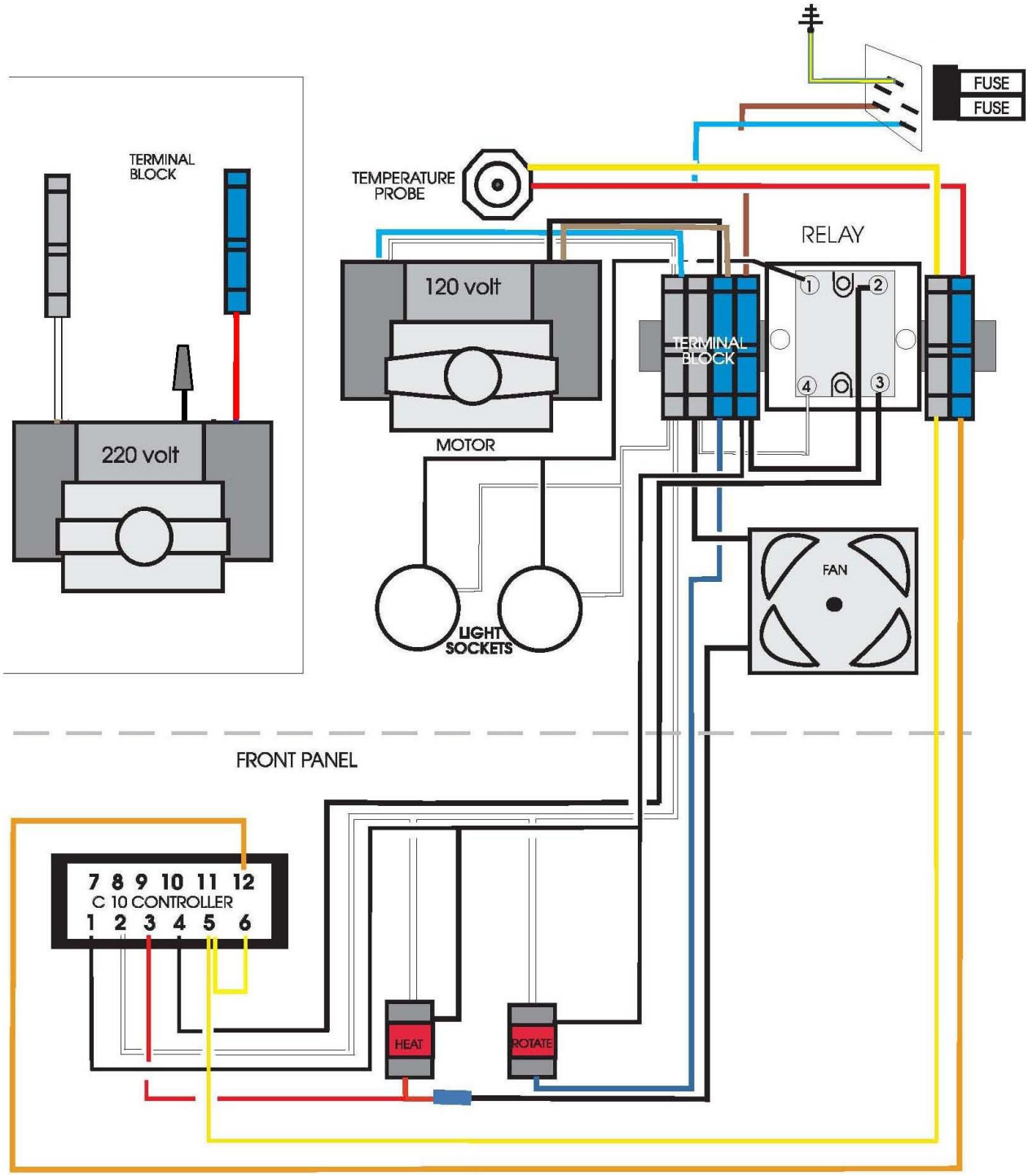
The Athena Controller (shown above) maintains the proper temperatures within the D200/D200I. To configure the controller, follow the procedure below. If you make a mistake at any point in the procedure, go back to step 2 and start over.

1. Power on the instrument. The current temperature will show on the display.
2. Press **tab** until you see PASS on the display.
3. Press ▼ ▲ until you see 33 on the display. Press ↵ You will see ConF on the display.
4. Press ▼ ▲ until you see 0000 on the display. Press ↵ You will see Unit on the display.
5. Press ▼ ▲ until you see °C on the display. Press ↵ twice to see CodE on the display.
6. Press **tab** . You will see the current temperature show on the display.
7. Press **tab** twice to see tunE on the display.
8. Press **enter** . You will see SL.u on the display.
9. Press **enter** . You will see SL.d on the display.
10. Press **enter** . You will see S.P.L on the display.
11. Press ▼ ▲ until you see 0.0 on the display. Press ↵ You will see S.P.H on the display.
12. Press ▼ ▲ until you see 45.0 on the display. Press ↵ You will see t.F.iL on the display.
13. Press **tab** . You will see PASS on the display.
14. Press **tab** . You will see the current temperature show on the display.
15. Press ▼ ▲ until you see 39.5 on the display.

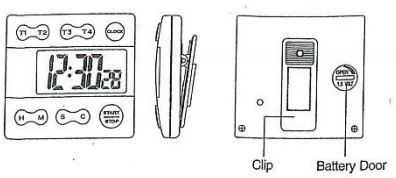
Let the controller sit for 30 seconds. The temperature is now set to 39.5 and the menu settings are complete.

Appendix D – Daisy Wiring Diagram

Inlet Power: 100 – 120V
220 – 240V



Appendix E- Timer Operating Instruction



Product Features:

1. Jumbo display 4 channel LCD count-down / count-up timer with clock and alarm features.
2. 6 digit display shows hour, minute and second setting for timers and clock.
3. Automatic count-up after timer counts down to zero.
4. Count-down timer: Maximum setting is 99 hours, 59 minutes, and 59 seconds. Counts down at 1 second resolution. Count-up timer: Maximum count-up range is 99 hours, 59 minutes, and 59 seconds. Counts up at 1 second resolution.
5. Memory recall function for count-down timers.
6. Timer alarm sounds for 1 minute when timer counts down to zero.

Clock Mode:

1. Press the clock button to enter clock mode. Preset time (hours, minutes and seconds) and flashing colon are displayed.
2. Press and hold START/STOP button for 3 seconds to toggle 12/24 hour format.

Clock Setting Mode:

1. Press and hold the clock button for 3 seconds (until beep) to enter time setting mode. "HOUR", "MINUTE", "SECOND" and colon flash on display. The "P" indicator shows in 12 hour format.
2. Press the HOUR button to advance hour setting. Press and hold for 2 seconds for fast setting.
3. Press the MINUTE button to advance minute setting. Press and hold for 2 seconds for fast setting.
4. Press the SECOND button to reset second digit to zero when the second digits are within 00-29 second range. Press S button to reset second digits to zero and minute digits advance by 1 increment when second digit are within 30-59 second range.
5. When the time setting for the clock is ready, press the clock button once to return to normal clock display mode.

   **When the timer is running, the corresponding indicator (T1, T2, T3, T4) flashes on display. All four timers can run at the same time. When the timer reaches to 0:00 00, the buzzer will sound and the corresponding indicator (T1, T2, T3, T4) will flash relatively slower. More than one indicator can flash at the same time.

Count Down Timer Setting:

1. Press the T1, T2, T3, or T4 button enter to the desired timer channel. In timer mode, the colon does not flash and the corresponding timer indicator "T1", "T2", "T3", and "T4" shows on display.
2. Press the HOUR button to advance hour digit.
3. Press the MINUTE button to advance minute digit.
4. Press the SECOND button to advance second digit.
5. Press the HOUR, MINUTE or SECOND button for 2 seconds for fast setting of the corresponding digit.
6. Press the CLEAR button to clear the Count-Down Timer and the corresponding timer memory to 00H00M00S
7. Press the HOUR and the CLEAR buttons simultaneously to clear the hour digit setting only.
8. Press the MINUTE and the CLEAR buttons simultaneously to clear the minute digit setting only.
9. Press the SECOND and the CLEAR buttons simultaneously to clear the second digit setting only.

Count-Down Timer START/STOP

1. After time setting is ready, press the START/STOP button once. The timer will start to countdown at 1 second resolution.
2. Press the START/STOP button once to stop the counting timer.
3. Press the START/STOP button once again, the timer will resume counting.

Count-Down Timer Alarm

1. When the timer counts down to 0:00 00 in its timer mode, the buzzer will sound.
2. When the timer counts down to 0:00 00 but not in its timer mode, the buzzer will sound and the flashing frequency of corresponding indicator is relatively slower.
3. When two timers countdown to 0:00 00 at the same time, the timer which shows on display will sound and the other's indicator will flash relatively slower.
4. Press any button to stop timer alarm and the count-up timer.

Count-Down Memory Recall

1. Press the START/STOP button to recall previous timer setting. Press the START/STOP button again to start the timer.

Stop Watch Mode

1. In timer mode, clear timer by pressing the CLEAR button.
2. Press the START/STOP button to start the stop watch counting up at 1 second resolution.
3. Press the START/STOP button to stop counting up.
4. When timer counts up to 99H 59M 59S it starts to count up again from 00H 00M 00S.

Battery Replacement

Use a coin to open the battery cover at back side of timer, following the arrow direction. Remove exhausted battery and insert a new 1.5V G-13 size button cell battery (make sure positive '+' is facing up) and close battery cover.

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Automation saves time and money!

ANKOM Technology is an international company with products that include…

	**FLEX Analyte Extractor** • 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
	**TDF Dietary Fiber Analyzer** • Automates AOAC 991.43, 985.29, 2009.01, and 2011.25 (and associated AACC methods) • IDF/SDF and TDF values • Faster, Technician-free Filtering • Computer controlled operation Reduced per assay costs
	**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 • Anaerobic activity analyses (rumen, yeast, beer/wine fermentation, biomass, biodegradability, etc.) • Soil respiration • Wireless Computer control and data storage

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