

Operator's Manual

Daisy^{II} Incubator

D200, D200I



ANKOM
TECHNOLOGY

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Congratulations on your purchase of an ANKOM Technology Daisy^{II} Incubator.

Your selection of an ANKOM system indicates that you are looking to increase your wet chemistry efficiency and maintain the highest levels of precision and accuracy possible.

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 ANKOM 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.

ANKOM Technology is committed to your total satisfaction. We are always available to assist you get the most out of your ANKOM products. Feel free to contact us with your questions and suggestions. 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.

Safety Precautions



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



Rear Surface:

Caution, 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. Electrical shock or electrocution may result if ignored.

General:

-All switches should be in the off position before plugging the power cord into the electrical outlet. The **Rotate** and **Heat** switches are in the **OFF** position when they are pressed down.

- An electrical fuse will disconnect the electrical power supply in case of malfunction.

Specifications:

DAISY^{II} (D200)	120V~ 3 amp 60Hz
DAISY^{II}-(D200I)	230V~ 2 amp 50Hz

Environment Operating Range:

Temperature: 15-30°C (60-85°F)
Humidity: 20-60% RH

WARRANTY

ANKOM Technology warrants the ANKOM DAISY^{II} Incubator against any defects due to faulty workmanship or material for a one-year period after the original date of consumer purchase. This warranty does not include damage to the instrument resulting from neglect or misuse. If the instrument is damaged as a result of defects in the workmanship or materials during the warranty period, ANKOM Technology will repair or replace the instrument free of charge. **Glassware is not covered under warranty.**

For questions regarding the warranty or instrument, please contact ANKOM Technology at

PHONE: 315 986-8090

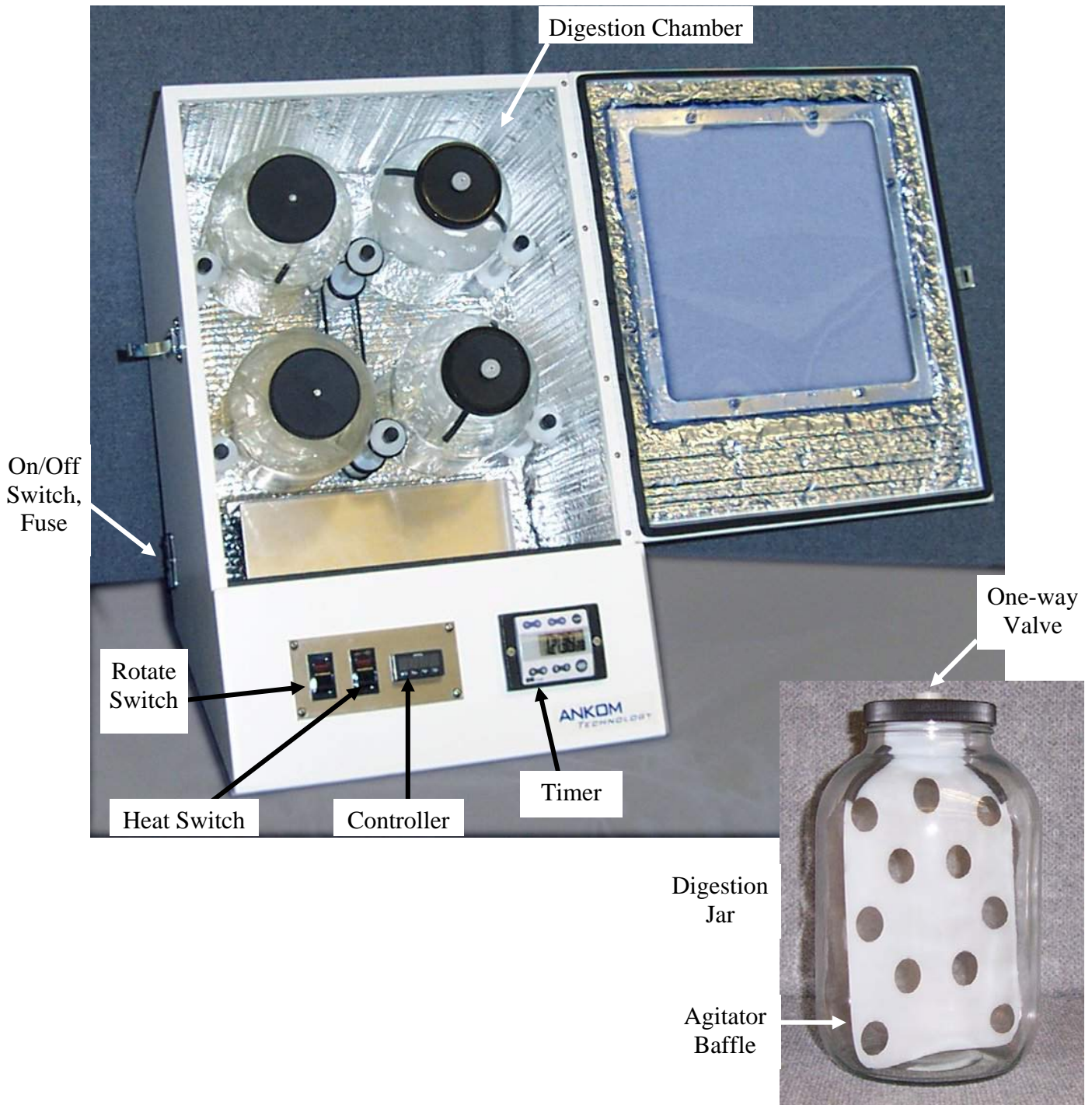
FAX: 315 986-8091

service@ankom.com

Instrument Description and Set-up:

Your instrument comes complete with a power cord and digestion jars.

- Set the instrument on a firm, level surface. Place the back of the instrument no closer than one inch from a wall. **NOTE:** Do not locate this instrument where it will be subject to excessive shock, vibration, dirt, moisture, oil, or other fluids.
- Plug the power cord into the plug outlet and then into an electrical outlet.
- The **Rotate** button controls the rotation of the digestion jars.
- The **Heat** button allows the controller to heat the digestion jars, samples, and rumen inoculum.
- The **Controller** maintains the temperature inside the chamber at $39.5^{\circ}\text{C} \pm 0.5$. Do not adjust without consulting ANKOM Technology.



Operation

Your **DAISY^{II}** Incubator is designed to provide incubation and agitation of samples at a constant 39.5C+/-0.5. 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 **Main Power Switch** on.
- 2) Add samples and solution to digestion jars as per procedure.
- 3) Turn the **Heat** and **Rotate** buttons on. Visually confirm the jars are rotating and the heat lamps are on.

To End the Cycle:

- 1) Turn the **Heat** and **Rotate** buttons off.
- 2) Empty 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 drive belt clean the outside of the jars with window cleaner.

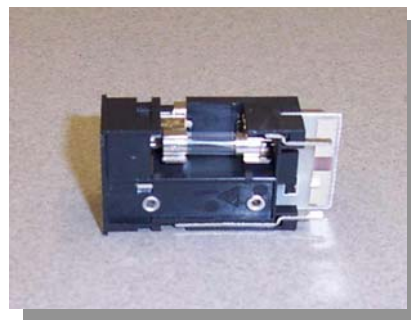
Troubleshooting

Your ANKOM instrument has been designed for long service and ease of maintenance. Most problems are easily diagnosed and fixed. If you experience problems that are not addressed in the information below or if you have any questions, contact ANKOM Technology for assistance. We are committed to fast and reliable service.

Fuse Replacement:

Replace both fuses- 120V(10amp) or 220V(5 amp). Install the Fuse Holder with the correct voltage right side up.

Pry Fuse Holder out of Power Switch.



This is oriented for 220v operation.



Problems:

Problem: The jars are slipping.

Cause: Jars are dirty; belts are positioned poorly, not enough friction between jars and rollers.

Solution: Clean and dry jars. Check belt positioning. Apply white surgical tape to the roller contact areas of the jars. The belt can be twisted in a figure "8" to increase belt tension.

Troubleshooting (Continued)

Problems (Continued):

Problem: The temperature controller reading does not match the actual temperature. The temperature controller reading is low.

Cause: A bulb is not working, the air flow is obstructed.

Solution: Make sure both heat bulbs are working. Check for an obstruction of hot air flow. Make sure the Daisy is not under a cold air vent. If this does not solve the problem, contact ANKOM Technology.

Problem: Controller reads “no load.”

Cause: A power surge has occurred.

Solution: Reprogram your controller using the controller menu setup sheet in the Appendix B.

Problem: Low Digestibility values.

Cause: Sample size is too large, bags were not acetone rinsed, the rubber baffle spacers fell off

Solution: Ensure that the procedure is followed completely. Reduce sample size to .025 per procedure, check baffle spacers, rinse bags with acetone and thoroughly dry prior to filling.

Note:

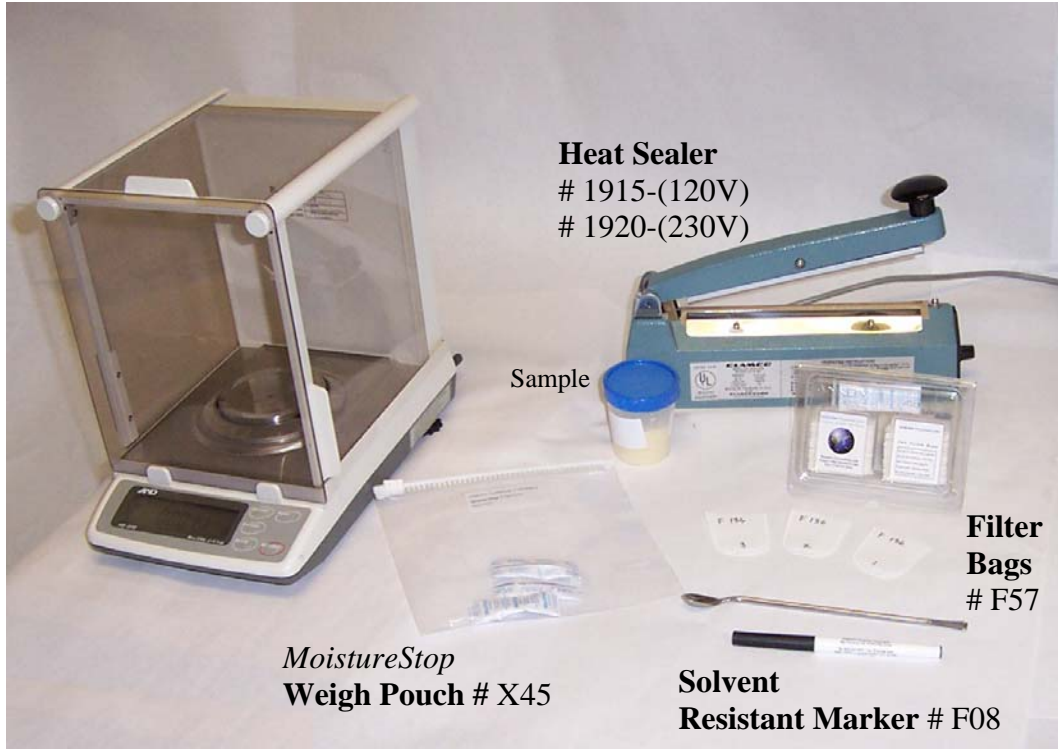
For problems relating to the results of your analyses using the ANKOM DAISY^{II} Incubator, first check to ensure the procedures sent with your machine were followed. Some possible variables that may affect your results are:

- Improper solution concentrates.
- The assay procedure was not carefully followed.
- Oven drying temperatures were too high (drying temperatures higher than 105° C may affect results).
- Improper calculation of blank bag correction or using the wrong values in the formula.
- Modifying the settings on the temperature controller.

**W-1 ANKOM Technology Fiber Weighing Procedure
For In Vitro Determinations**

Electronic Balance-

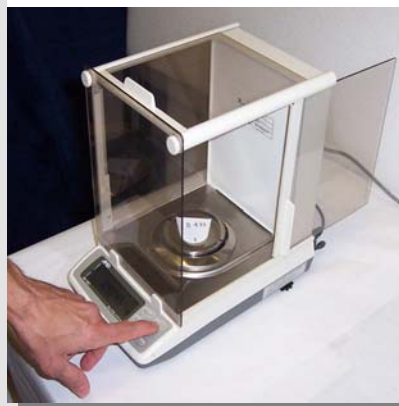
We recommend a four place readout on the balance and suggests “Balance Talk™” or other LIMS software for data input and management.



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



2. Weigh and record filter bag weights.



3. Tare weight of filter bag and Bag Holder.

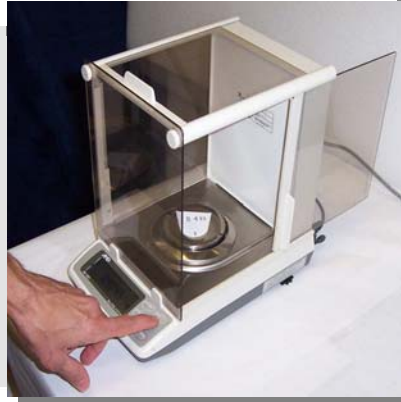


W-1 ANKOM Technology Fiber Weighing Procedure (Continued)
For In Vitro Determinations

4. Add 0.25g to .05g of sample to bag using a spatula. Be careful not to get sample particles on top edge of the bag sealing area.



5. Weigh sample and record weight



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



7. Seal each filter bag no more than 4mm from it's open end. Hold down handle for 2-3 seconds after the red light turns off.



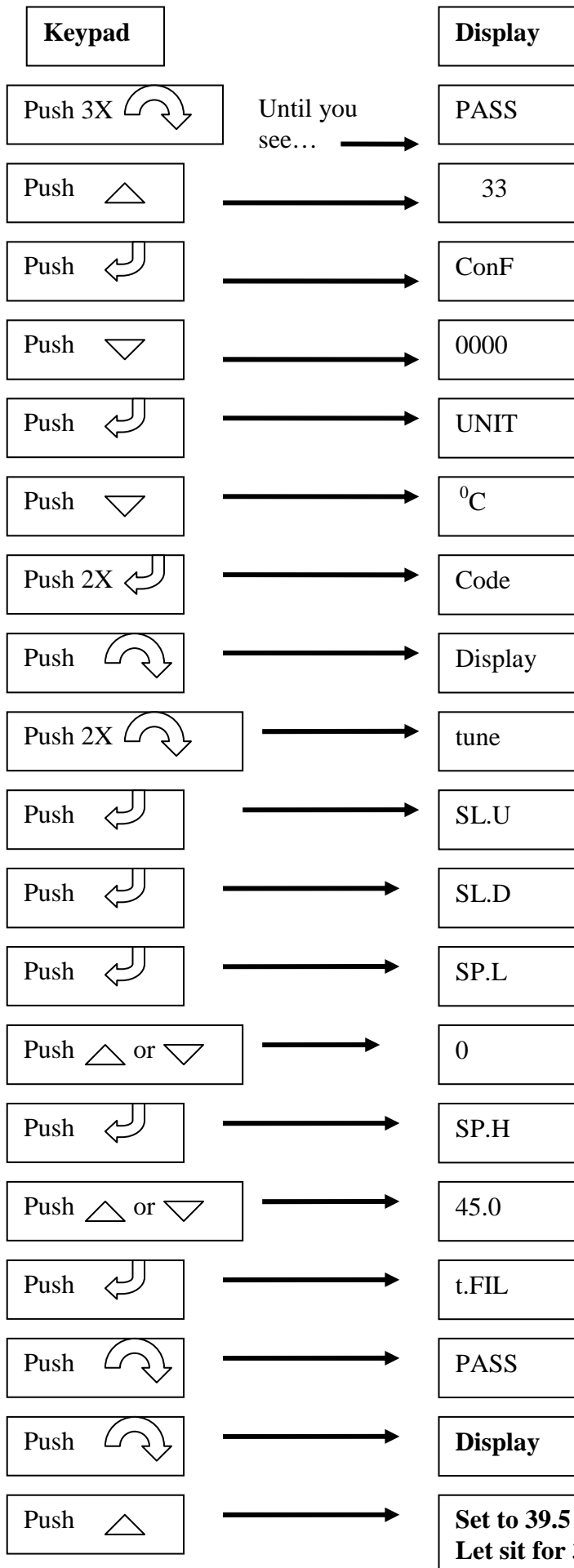
8. The seal can be seen as a solid melted stripe along the top edge.



9. Perform the extraction as per procedure.

10. After digestion is complete, acetone-rinse and dry according to procedure. Remove from oven and immediately store in the *MoistureStop* Pouch
11. Cool to room temperature and re-weigh each bag.





ANKOM Technology Daisy C 10 Controller Menu Set Up

Follow the instructions to enter the controller settings. If you are unable to install the settings, please call Customer Service at (315- 986-8090 or contact us at service@ankom.com)

Appendix C

Daisy Assemblies and Parts

D1.5

Temperature Probe

1 LB



D2

Jar Assembly

3 LB



D2.5

Jar Lid

1 LB

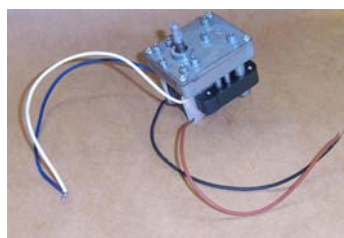


D6

120 Motor

Instructions-D6/7

4 LB

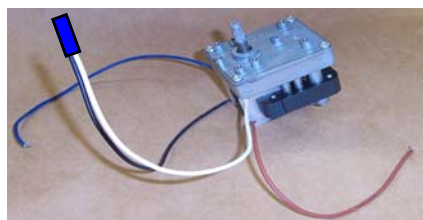


D7

220 Motor

Instructions-D6/7

4 LB



D13

XT32 Controller

Instructions-D4

1 LB



F 17

Timer

1 LB



Daisy Assemblies and Parts (Continued)

<u>Item</u>	<u>Shipping Weight In Pounds</u>
629– Relay 120V-	1
630– Relay 220V-	1
806– Bulb 220V	1
807– Viton Drive Belt-304 O-ring-	1
808– O-ring 322 Buna	1
814– Fan 120V	1
815– Fan 220V	1
822– Heat/Rotate Switch	1
816– Light Bulb Red 60W 120V	1
832– Roller 2A Idler	1
833– Roller 2B Drive Idler	1
834- Roller 2C Drive	1
835– Roller 2D Drive w/set screw	1
836– Roller E Pivot Bumper	1
851-Valves one way for the jars	1
853- 3.5A Fuse-Dual Voltage	1

ANKOM Technology Method 3

In Vitro True Digestibility using the DAISY^{II} Incubator
ANKOM TECHNOLOGY - 08/05

A. Reagents

(a) <u>Buffer Solution A:</u>	<u>g/liter</u>
KH ₂ PO ₄	10.0
MgSO ₄ •7H ₂ O	0.5
NaCl	0.5
CaCl ₂ •2H ₂ O	0.1
Urea (reagent grade)	0.5
(b) <u>Buffer Solution B:</u>	
Na ₂ CO ₃	15.0
Na ₂ S•9H ₂ O	1.0

(c) Neutral Detergent Solution

B. Apparatus

- (a) DAISY^{II} Incubator
- (b) Filtration device - F57 Filter Bags.
- (c) Impulse bag sealer - 1915/1920 Heat Sealer.
- (d) Thermos
- (e) ANKOM^{200/220} Fiber Analyzer

C. ProcedurePreparation 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 (W₁). Zero the balance and weigh 0.25g of sample (W₂) **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 (C₁).

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 CO₂ 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 CO₂ 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 CO₂ gas for thirty seconds and secure lid.
- d) Repeat process for all digestion jars to be used. NOTE: Do not allow CO₂ gas to bubble through the buffered inoculum, rather use the CO₂ 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 thoroughly with cold tap water until water is clear. Use a minimum of mechanical agitation.
- 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 **ANKOM²⁰⁰ Fiber Analyzer** and follow the procedure for determining NDF. Record the post *in vitro* NDF weight as W₃. NOTE: Bags can be stored in the refrigerator or freezer until NDF determinations can be performed.

D. Calculate:

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

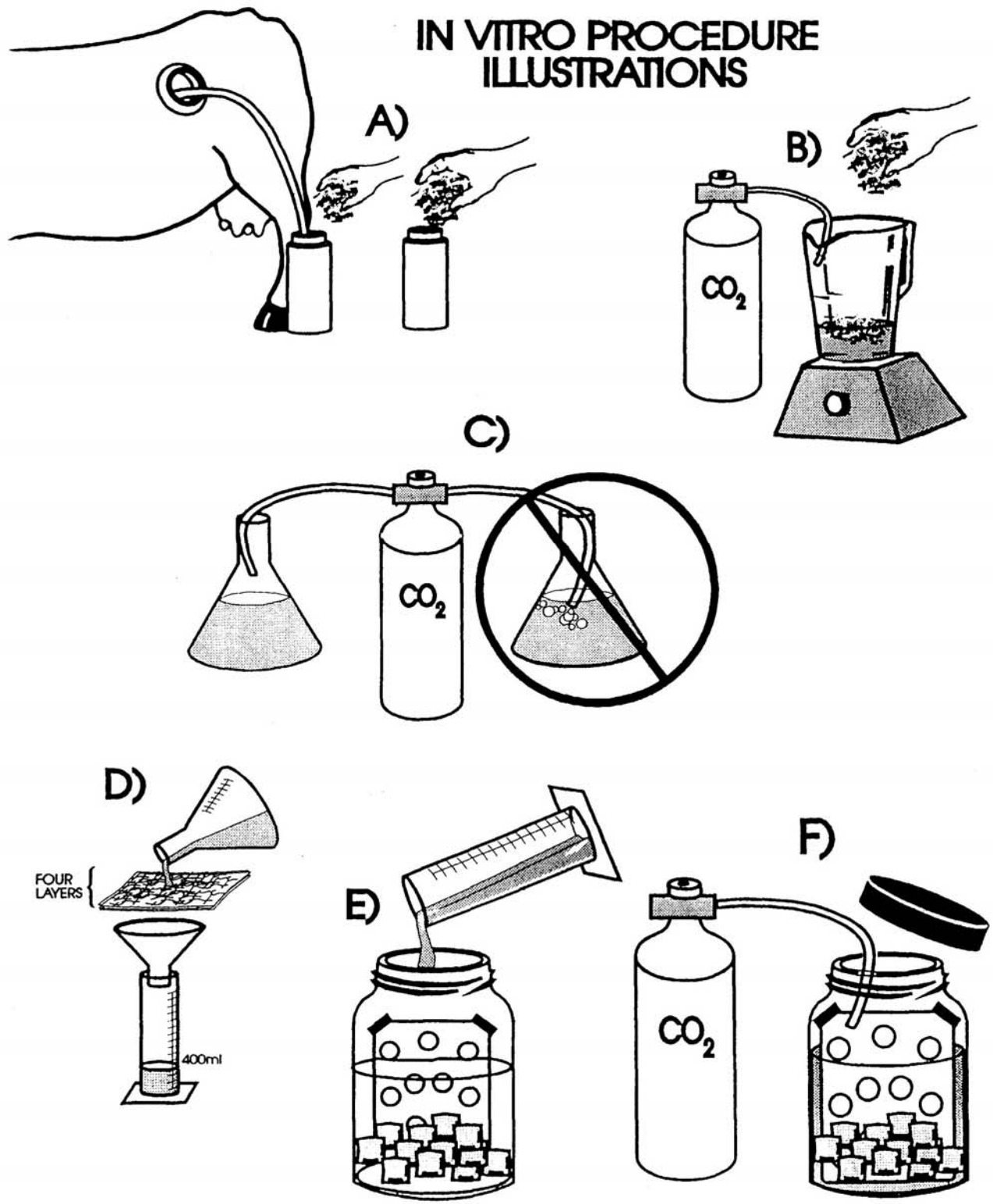
$$\% \text{ IVTD}_{\text{DM}} \text{ (DM basis)} = \frac{100 - (W_3 - (W_1 \times C_1))}{(W_2 \times \text{DM})} \times 100$$

Where:

- W₁ = Bag tare weight
- W₂ = Sample weight
- W₃ = Final bag weight after In Vitro and sequential ND treatment
- C₁ = Blank bag correction (final oven-dried weight/original blank bag weight)

Appendix D (continued)
Assay Procedures

**IN VITRO PROCEDURE
ILLUSTRATIONS**



DAISY WIRING DIAGRAM

11 14 03

16

INLET
POWER
120/60 Hz
220/50 Hz

