pH and Acidity in Kombucha

(as Lactic Acid)

DOC316.53.93141

Endpoint potentiometric titration
Scope and application: Kombucha
Titrant calibrated using Standardettes[1]
(Based on ASBC Methods of Analysis—Beer-8)

This application note covers the following application:

Sample Type	pH Endpoint	Primary Results	Titrant Calibration	Syringe	ID	Application Name
Kombucha	8.2	Total Acidity as mL 1 N Alkali per 100 g Total Acidity as % Lactic Acid	Standardettes	Syringe 1 (T1)	1B	1B-pH acidity Kombucha

1. Important Information

- This application records the initial sample pH and gives titration results as mL of 0.1 N NaOH titrant used to reach the endpoint, mL of 1 N NaOH used per 100 g, and Total Acidity expressed as **% Lactic Acid**.
- **This application uses a 25-mL syringe and the syringe volume setting must be changed on the instrument before use**. Refer to section 10.1 of the Appendix for instructions.
- This application uses H_2SO_4 Standardettes to calibrate the titrant. Alternatively, the titer may be entered from the C.O.A. Refer to section 10.3 of the Appendix for instructions.
- Highly acidic samples may need to be titrated in a larger beaker such as a 250-mL, low-form, polypropylene beaker (108046), so that the beaker does not overflow. Alternatively, the sample volume may be decreased to reduce the amount of titrant required. Be sure to change the sample volume in the “Application Edit Window” if a sample volume other than 50 mL is used. Instructions for application customization are included in section 10.4.1 of the Appendix.
- Kombucha must be degassed before titration. Refer to your laboratory SOP (Standard Operating Procedure) for details. A table of degassing methods is provided in section 10.5 of the appendix.
- Please review the appendix for important information on the following topics:
  - Changing the syringe volume—Required at startup (Appendix 10.1)
  - Calibrating the electrode (Appendix 10.2)
  - Changing the application for calibration of the titrant (Appendix 10.3)
  - Modifying the application files to accommodate specific requirements (Appendix 10.4)
- Hach has “draft level” SOPs that use a sequence of steps and pictures to describe the basic operation of the AT1000 instrument with these applications. These documents can be used for general training and as a template for lab SOPs and Work Instructions. If these documents are not included on your AT1000 USB flash drive, please request electronic copies from your local Hach Representative.

2. Introduction

This working procedure refers to the determination of Total Acidity in Kombucha. Total acidity (TA) in this application is defined as the number of acidic protons that the organic acid can potentially donate. It is experimentally determined by measuring the amount of sodium hydroxide that is required to raise the pH to 8.2, where neutralization of the acids occurs. Note, however, that all of the protons do not dissociate, so the application is more accurately defined as titratable acidity, which is the measurement of hydrogen ions consumed by titration with a standard base to an endpoint. However, titratable acidity is often used as an approximation of total acidity, and thus is generally referred to as TA.

[1] Standardette Solutions are products of Chemical Standards Laboratory.

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3. Principle

This kombucha application is based on a weak acid titration by a strong base to a pH of 8.2. The Total Acidity result is expressed as the % Lactic Acid to the specified endpoint. The application uses a 50-mL sample volume, but the volume can be edited if necessary (refer to section 10.4.1 of the Appendix). The mL of titrant used, sample pH, and mL of 1.0 N NaOH/100 mL are also displayed.

4. Electrode and reagents

Electrode	PHC72501 Intellical combined pH electrode with integrated temperature sensor
Titrant	NaOH, 0.1 eq/L. Use a commercial solution (19153) or dissolve 4.00 g of sodium hydroxide (18734) in 1 L of CO_2-free boiled water.
Standard	H_2SO_4 Standardettes (ATS100) for calibration of 0.10 N NaOH titrant
Buffer solutions	pH 4.01 (2283449), 7.00 (2283549), and 10.01 (2283649) buffers, color-coded
Deionized water	

5. Ranges and settings

5.1. Default parameters

The working procedure uses the following parameters:

- Sample volume = 50 mL
- Syringe volume = 25 mL

5.2. Working range

For most samples, one to three syringes (up to 75 mL) of titrant is sufficient to reach the equivalence point. However, the syringe can refill up to 6 times during a titration to analyze a highly acidic sample of kombucha.

The sample volume can be adjusted to obtain a sufficient equivalent volume without requiring too much time for syringe refilling or using large amounts of titrant. This change must be made in the application. Refer to section 10.4.1 of the Appendix.

Note: If using a smaller sample volume or a larger beaker, it may be necessary to add DI water to the sample beaker to cover the pH electrode and the delivery tip.

Important: **Highly acidic samples need to be titrated in larger beakers to prevent overflow.** If more than 75 mL of titrant is expected for the titration, use a larger beaker such as a 250 mL, low form, polypropylene beaker so that the beaker does not overflow.

5.3. Settings

5.3.1. pH in kombucha settings

Name	Default parameter	Unit
Probe		
Recommended probe	PHC72501	
Stirring		
Stirring speed	15	%
Time	0[1]	s
pH measurement		
Measured parameter		pH
Stability criteria	0.017	pH/min
Stability max time	300	s

[1] This application is designed for continuous stirring during the pH measurement (Time = 0). Adjust this setting if compliance with ISO standards is required (recommended Time = 15).

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5.3.2. Total acidity in kombucha settings (based on ASBC Method)

Name	Default parameter	Unit
Sample		
Name	Kombucha	
Amount	50[2]	mL
Amount min	45	mL
Amount max	55	mL
Electrode		
Recommended probe	PHC72501	
Titrant		
Name	NaOH	
Titrant concentration	0.1000	eq/L
Syringe	Syringe 1	
Application		
Stirring speed	30	%
Measured parameter		pH
Predose	7.0	pH
Max volume stop point	150	mL
Stop on last EQP	True	
Delay	10	s
Min increment size	0.1	mL
Max increment size	1.0	mL
EP Ordinates	8.2	pH
Result 1 name	Value	pH
R1 hide	No	
Result 2 name	NaOH used to EP	mL
R2 hide	No	
R2 resolution	3 decimals	
R2 min	0	mL
R2 max	150	mL
R2 QC min	0	mL
R2 QC max	150	mL
Result 3 name	Total Acidity	mL 1.0 N Alkali per 100 g
R3 resolution	1 decimal	
R3 hide	No	
Result 4 name	Total Acidity	% as Lactic Acid
R4 resolution	2 decimals	
R4 Hide	No	

[2] To use a sample volume other than 50 mL, change the sample volume in the application edit window (refer to section 10.4.1 of the Appendix).

5.4. Modification of the settings

The parameters can be adjusted in the application edit window to reach the best compromise between accuracy and titration time as well as to set up titrations for analysis of specific products. The original applications on the USB flash drive can be duplicated and edited (for example predose) to accommodate multiple sample types. See the appendix for further details.

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For high acidities requiring a high titrant volume, the titration time can be reduced with an addition of titrant (predose) at the beginning of the titration. In the application edit window, enter the predose volume (in mL) and the stirring time that follows the predose volume. Alternatively, the sample volume can be decreased. This change must be made in the application. Refer to section 10.4.1 of the Appendix.

6. Titration procedure

1. Rinse the pH probe with deionized water.
2. Add the degassed sample to an appropriately sized beaker.

   Important: *The applications as provided are for a 50-mL sample volume. If a sample volume other than 50 mL is used, be sure to change the sample volume in the application edit window. Refer to section 10.4.1 of the Appendix for details.*
3. Add a magnetic stir bar.
4. Dip the pH probe and the delivery tip into the solution. Make sure that the tip of the electrode does not hit the stir bar.

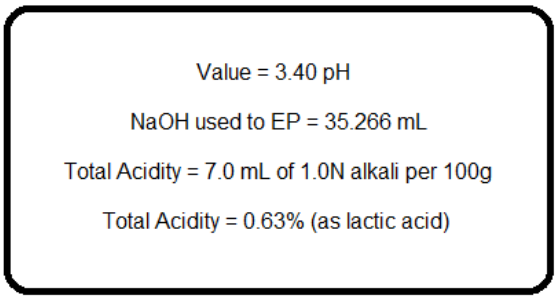
   Note: If using a smaller sample volume or a larger beaker, it may be necessary to add DI water to the sample beaker to cover the pH electrode and the delivery tip.
5. Start the application. You will see a prompt to enter the specific gravity of the sample.
6. Enter the specific gravity using the arrows on the titrator or a connected USB keyboard, then press **Select**. The titration continues to the pH endpoint.

   At the end of the titration, one window shows the results. A second window shows the titration curve, and a third window shows the titration curve, derivative curve, and the equivalent point coordinates.

   After the titration, there are two possible tasks:

   - Run a replicate analysis of the sample. The replicate is used to study the repeatability by analyzing several aliquots of the same sample successively. At the end of each titration, a window shows the average value, the standard deviation (SD) and the relative standard deviation (%RSD).
   - Analyze a new sample. Another titration can be started, but no Standard Deviation or %RSD value will be calculated.

   Results at the end of the titration are shown as follows:



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7. Results

7.1. Calculations

The calculations used in this application are:

$$\text{Total Acidity as mL of 1.0 N alkali per 100 g of kombucha} = \frac{(V1 \times 10)}{(SA \times G1)}$$

Where:

V1 = volume of 0.1 N NaOH used

SA = mL of sample

G1 = Specific Gravity of sample

$$\text{Total Acidity as \% Lactic Acid} = \frac{(V1 \times 0.9)}{(SA \times G1)}$$

Where:

V1 = volume of 0.1 N NaOH used

SA = mL of sample

G1 = Specific Gravity of sample

0.9 = Factor to convert mL of titrant to % Lactic acid

7.2. Selected experimental results

Three replicates of each sample were analyzed. Samples were degassed with nitrogen before titration.

Kombucha 1 (specific gravity 1.0)

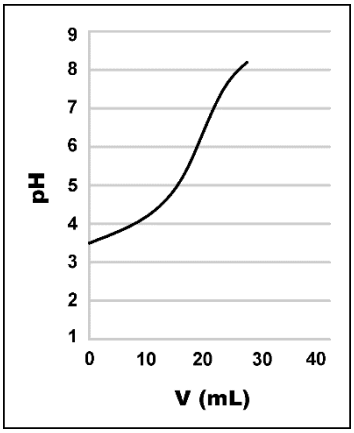
Parameter	Average	Relative Standard Deviation	%RSD
pH	3.08	0.02	0.59
NaOH used to EP (mL)	35.955	0.018	0.05
Total Acidity (mL 1.0 N alkali per 100 g)	7.2	0.00033	0.05
Total Acidity (% Lactic Acid)	0.65	0.0036	0.05

Kombucha 2 (specific gravity 1.02)

Parameter	Average	Relative Standard Deviation	%RSD
pH	3.36	0.0168	0.5
NaOH used to EP (mL)	17.62	0.029	0.18
Total Acidity (mL 1.0 N alkali per 100 g)	3.5	0.0063	0.18
Total Acidity (% Lactic Acid)	0.31	0.00056	0.18

7.3. Example of a titration curve

The titration curve shown below is the type of curve that is obtained during the sample analysis. The highest point is determined by the endpoint pH. The lowest point is determined by the predose volume (or pH ordinate).



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8. Recommendations

Always rinse the pH probe and the delivery tip between measurements.

Refill the electrode regularly with KCl saturated solution to maintain the level of the internal solution at approximately 1 cm (0.4 inches) below the refill hole.

Calibrate the electrode daily.

Calibrate the titrant daily, when a new bottle is started or when fresh titrant is prepared.

9. Bibliography

- *ASBC Methods of Analysis—Beer-8*

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10. Appendices

10.1. Changing the syringe volume on the AT1000—Required at startup

The AT1000 instrument is shipped from the factory with the syringe volume set to 10 mL. The beverage applications require a 25-mL syringe volume. The syringe volume must be changed before the applications can be run. Complete the steps that follow to make this change:

1. From the Home screen select **Maintenance**>**Syringe Management**>**Syringe volume change**.

   Note: If your AT1000 instrument has 2 syringes, select the syringe to edit.

2. Use the arrow keys to highlight **25mL (Hamilton)** and press **Select**. The display shows "Applying 25mL (Hamilton) settings" followed by "Syringe volume updated".
3. Press **OK**.
4. Press **HOME** to return to the Home screen. The AT1000 is ready to run the beverage applications with the 25-mL syringe.

10.2. Electrode calibration

Daily calibration of the electrode is strongly recommended for the precision of the titration. It is recommended to calibrate the probe with at least two pH standards that are at the same temperature as the samples. Complete the steps that follow to calibrate the electrode:

1. Pour a sufficient amount of the first buffer solution into a 50-mL beaker. Add a magnetic stir bar.
2. Put the beaker under the AT1000 probe holder. Dip the probe in the buffer solution.
3. Start the electrode calibration sequence. Make sure that the stir bar does not hit the delivery tip or the electrode.
4. When the measurement is complete, remove the buffer solution and rinse the probe.
5. Repeat the above steps for each buffer solution.
6. At the end of the calibration, review the slope and offset, then accept or reject the calibration.

Default settings for electrode calibration

Name	Setting	Unit
Stirring speed	25	%
Stability criteria	0.017	pH/min
Stability max time	300	s
Slope limit min	94	%
Slope limit max	104	%
Offset limit min	44	mV
Offset limit max	74	mV

10.3. Titrant concentration

The concentration of the sodium hydroxide titrant can be determined from a titration with an H_2SO_4 Standardette (section 10.3.1). For commercial NaOH solutions, the concentration that is shown on the vendor C.O.A. can be entered through the edit application window (section 10.3.2). Refer to your laboratory SOP for guidance on which determination is appropriate for your application.

10.3.1. Calibration procedure using Standardettes

1. Remove the cap from the Standardette.
2. Carefully remove the blind plug.
3. Rinse the inside and outside of the plug into the titration beaker with 5 to 10 mL of water.
4. Empty the contents of the Standardette into the titration beaker.
5. Rinse the inside of the Standardette into the titration beaker five times with water.
6. Add a stir bar and put the beaker under the AT1000 probe holder.

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7. Dip the probe and the dispensing tip into the solution.
    - Be sure that the NaOH dispensing tip and the pH probe are submerged in the sample and that the stir bar does not hit the end of the pH probe.
    - Add DI water if necessary.
8. Start the titration.
9. When the titration is complete, enter the concentration value for the Standardette as follows:
    - When prompted to enter the weight, find the milliequivalents value that is shown on the box. **DO NOT USE THE GRAMS VALUE.**
    - Use the arrow keys to enter the milliequivalents value.
    - When the correct value is entered, press **Select**.

Default settings for titrant calibration with Standardettes

Name	Default parameter	Unit
Titrant		
Name	NaOH	
Titrant concentration	0.1000	eq/L
Syringe	Syringe 1	
Standard		
Name	Standardette	3.8–4.2
Amount	Varies—Enter value from label	
Amount min	3.800	
Amount max	4.200	
Molar weight	100*	g/mol
EP titration		
Stirring speed	25	%
Measured parameter		pH
Predose	30	mL
Max volume stop point	50	mL
Stop on last EQP	True	
Delay	10	s
Min increment size	0.08	mL
Max increment size	0.8	mL
EP Ordinate	7.0	pH
Result name	Titer	
Result resolution	4 decimals	
Result min	0.0900	eq/L
Result max	0.1100	eq/L

* Do not change! Used in the titer calculation.

10.3.2. Entering the titer from a C.O.A.

1. From the Home screen, select **Settings**>**Applications.**
2. Select **Edit** from the list of actions.
3. Highlight the application and press **Edit.**
4. Scroll down to **Titrant**.
5. Select **Real Concentration**.

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6. Use the arrow keys or a connected USB keyboard to enter the titer value from the C.O.A.
7. Select **OK**.
8. Press **HOME** to return to the Home screen.

10.4. Customization of the Kombucha Application

One application for Total Acidity in Kombucha is included on the AT1000 USB flash drive. This application (listed in the table at the beginning of this application note) is designed to use 50 mL of sample as described in ASBC method Beer-8. The application can be customized in the Edit Application Window on the AT1000 to match the needs of each customer site (to accommodate the acidity of multiple products).

10.4.1. Simple application customization

Duplicating an Application

When customizing an application, it is best to make a copy of the original version before making changes. Below is the process for duplicating an application:

1. From the Home screen, select **Settings**>**Applications.**
2. Select **Duplicate** from the list of actions.
3. Use the arrow keys to select the checkbox next to the application(s) to duplicate.
4. Select **Duplicate** and then **OK** after the screen shows “successfully loaded”. The duplicated application appears in the application list.

   Example: A copy of 1B-pH acidity Kombucha appears as 1B-pH acidity Kombucha (2).
5. Select **Edit** from the list of actions on the Application settings screen.
6. Highlight the duplicated application and select **Edit.**
7. Use the arrow keys or a connected USB keyboard to edit the name of the duplicated application.
8. When the name is complete, select **OK**.

Changing the Sample Volume

This application was designed to use a 50-mL sample, but the sample volume can be changed in the Edit Application Window if a different sample volume is required.

1. From the Home screen, select **Settings>Applications**.
2. Select **Edit** from the list of actions.
3. Highlight the application and press **Edit.**
4. Use the down arrow to go to **Amount** under the Sample heading.
5. Select **Edit.**
6. Enter the desired sample volume and select **OK.**

Changing the Predose Ordinate

Predose is used to improve sample throughput (speed of titration) and can be adjusted as follows:

1. From the Home screen, select **Settings**>**Applications.**
2. Select **Edit** from the list of actions.
3. Highlight the application and press **Edit.**
4. Use the down arrow to go to **Predose Ordinate** under the Method heading.
5. Select **Edit.**
6. Enter the desired predose ordinate and select **OK.**

Hide or Show a Result

1. From the Home screen, select **Settings**>**Applications.**
2. Select **Edit** from the list of actions.

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3. Highlight the application and press **Edit.**
4. Use the down arrow to go to **Method**, then **Results**.
5. Select **Yes** or **No** to show or hide results:
   - **Yes**—the result is not shown (hidden) at the end of the titration
   - **No**—the result is shown at the end of the titration

10.4.2. Expert application customization (using Expert Mode)

NOTICE: Expert Mode gives the user access to critical parameters that if changed can cause the application to run incorrectly! Be sure to turn off Expert Mode after changes are complete.

Most adjustments can be made from the Standard Edit Application Window, but some initial customization may need to be completed in Expert Mode (changing the endpoint, for example). Expert Mode gives access to critical instrument parameters. Changes other than those listed below should not be made without contacting Hach technical support.

Change Predose from Ordinate to Volume

1. From the Home screen, select **Settings**>**Options**>**Expert Mode**.
2. Press **HOME** to return to the Home screen.
3. Select **Settings**>**Applications**>**Edit**, then select the application to be changed.
4. Read the warning and select **OK**.
5. Scroll through the application to find the section on predose.
   - **Do not change any settings related to the electrode or related to detection. Any changes can have adverse effects on the result.**
   - A delay of 20–30 seconds following the predose allows equilibration of the sample with the initial larger dose of titrant prior to the start of the titration.
   - A stirring speed of 20–40% is typical.
6. Highlight **Predose Type** and select **Edit**.
7. Select **Volume**.
8. Enter the volume for the Predose.
9. Press **HOME** to return to the Home screen.
10. Turn off Expert Mode.

    Note: Expert mode also turns off when the instrument is turned off.
11. Run your revised application.

Change the Titration Endpoint

1. From the Home screen, select **Settings**>**Options**>**Expert Mode**.
2. Press **HOME** to return to the Home screen.
3. Select **Settings**>**Applications**>**Edit**, then select the application to be changed.
4. Read the warning and select **OK**.
5. Scroll through the application to find the section on **Ordinate Stop Point** and **EP 1 Ordinate**.

   Do not change any settings related to the electrode or related to detection. Any changes can have adverse effects on the result.
6. Use the arrow keys or a connected USB keyboard to change the **Endpoint pH**.

   *Note: The **Ordinate Stop Point** is set to 10.0 and should not be changed.*
7. Press **HOME** to return to the Home screen.
8. Turn off Expert Mode.

   Note: Expert mode also turns off when the instrument is turned off.
9. Run your revised application.

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Change the buffer set to IUPAC or DIN Buffers for electrode calibration

1. From the Home screen, select **Settings**>**Options**>**Expert Mode**.
2. Press **HOME** to return to the Home screen.
3. Select **Settings**>**Applications**>**Edit**, then select the application to be changed.
4. Read the warning and select **OK**.
5. Scroll through the application to find the section on Calibration mode.

   Do not change any settings related to the electrode or related to detection. Any changes can have adverse effects on the result.
6. Select **Fixed, Auto or Manual** and the number of buffers.

   Note: At least two buffers are recommended for the electrode calibration.
7. Select the first buffer and scroll to find the buffer set option: Colored, IUPAC, or DIN.
8. Select the desired buffer set.
9. Press **HOME** to return to the Home screen.
10. Turn off Expert Mode.

    Note: Expert mode also turns off when the instrument is turned off.
11. Run your revised application.

10.5. Degassing Applications and Alternatives that can be used for Kombucha

Application	Procedure	Time	Residual CO_2	Pros and Cons
Beer-1 A	Add the sample to a 500-mL Erlenmeyer flask. Shake by hand until there is no more gas in the sample.	5 to 20 minutes	Moderate to low (0.2 to 0.06)	The quantity of residual CO_2 depends on time and vigor of shaking.
Beer-1 D (Rotary Shaker)	Add the sample to a 500-mL wide-mouth Erlenmeyer flask. Put the sample in a rotary shaker for 12 minutes.	12 minutes	Low	Very specific in procedure with uniform results.
Pouring[1]	Pour the sample back and forth between two 500-mL beakers until there is no more gas in the sample.	5 to 20 minutes	Moderate to low (0.2 to 0.01)	The quantity of residual CO_2 depends on time and amount of pouring
Ultrasonic Bath[1]	Put the sample in the ultrasonic bath until there is no more gas in the sample.	15 minutes	Higher (0.529 vol)	The least effective of all applications but the easiest to process large numbers of samples.
Filtration	Filter the sample with fluted filter paper.	10 to 15 minutes	Moderate (0.41)	—
Ultrasonic Bath and Filtration[1]	Put the sample in the ultrasonic bath, then filter the sample with fluted filter paper.	10 minutes ultrasonic bath; 10 to 15 minutes filtration	None	More effective than ultrasonic bath or filtration alone, still higher.
Gas Purging	The sample has other inert gas bubbled through it to remove CO_2.	25 minutes	None	Not automated and is time-consuming.
Membrane Degassing[1]	The sample goes through an instrument that contains a semi-permeable membrane with negative pressure on one side to remove CO_2.	30 to 40 mL/min; 15 to 20 minutes for a complete sample	None	Not automated.
Water Vacuum Pump[1]	Put the sample under a water vacuum to remove CO_2 from negative pressure.	5 to 10 minutes	None	Not automated, removes other volatiles.
Microwave	Put the sample in a microwave to remove CO_2.	15 to 20 seconds	Low	Faster and more effective than filtration and ultrasonic bath.

[1] These degassing applications are usually used in the industry and found in the literature. However, these applications have not been validated through normal ASBC processes.

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11. Consumables and Replacement Items

Description	Quantity Per Test	Unit	Item Number
0.1 N NaOH Titrant	varies	1 L	19153
H_2SO_4 Standardette for titrant calibration	1	12/pkg	ATS100
pH 4.01 buffer for electrode calibration, color-coded, 500 mL	varies	each	2283449
pH 7.00 buffer for electrode calibration, color-coded, 500 mL	varies	each	2283549
pH 10.01 buffer for electrode calibration, color-coded, 500 mL	varies	each	2283649
Saturated KCl filling and storage solution for the PHC725 Red Rod Intellical Electrode	varies	59 mL	25118026
Beaker, plastic, 150 mL	1	5/pkg	LZE109
Beaker, plastic, 50 mL	1	5/pkg	LZE108
Beaker, plastic, 250 mL	1	6/pkg	108076
Beaker, plastic, 250 mL	1	each	108046
Magnetic stir bars, 20 x 6 mm	1	5/pkg	LZE136
PHC725 Red Rod Intellical pH electrode (replacement)	1	each	PHC72501
25-mL syringe		each	LZE103
Holder for 25-mL syringe		each	LZE107
Sodium hydroxide, ACS		500 g	18734

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