

**STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PLANT**

TITLE: pH Measurement and Calibration Operating Procedure

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A. Scope

This procedure describes how to calibrate a pH probe and take a measurement.

B. Safety and Training Requirements

Refer to UF lab safety policies and review the Material Safety Data Sheets (MSDS) for each material listed in section D below before starting any process work.

Review the location of fire extinguishers, fire blankets, safety showers, spill cleanup equipment and protective gear before beginning any process work.

During operations in the plant, the following safety gear will be utilized at all times:

- Lab Coat
- Safety Goggles or Face Shield
- Protective Gloves (nitrile, neoprene)
- Autoclave gloves

Avoid inhalation of vapors and wear nitrile or neoprene rubber gloves. Contain spills by using spill kits next to fermentors.

C. Related Documents and SOPs

1. Culture Sampling SOP
2. Denver Instrument 200 Series Meters Operating Manual
3. UF Biosafety manual

D. Preparation/Materials/Equipment

The chemicals/materials used in this SOP are listed below:

1. Denver Instrument Model 220 pH conductivity meter and probe.
2. Squirrt bottle filled with deionized water.
3. 3 ml transfer pipet
4. Aluminum foil covered glass bottle
5. 37% (w/w) formaldehyde (Fisher Scientific, F79-4, 4 L)
6. pH 7.0 buffer solution (Fisher Scientific, SB 108-20, 20 L)
7. pH 4.0 buffer solution (Fisher Scientific, SB 98-20, 20 L)
8. 100 ml graduated cylinder
9. 2 M Potassium chloride (KCl; Acros, 42409-0010, 1 kg)
10. Stir bar
11. Stir plate
12. 100 ml plastic beaker

E. Detailed Procedure

1. Prepare 2 M KCl by adding 596.48 g of KCl to a total volume of 4 liters.
2. Add 80 ml of 2 M KCl to a 100 ml graduated cylinder.
3. Squirrt 1 ml of 37% (w/w) formaldehyde into the graduated cylinder.

4. Store pH probe in the 2 M KCl formaldehyde solution containing cylinder.
5. Press the “Mode” key and select “1-pH.”
6. Press the “Standardize” key and “3-clear buffers to clear buffers.”
7. Press “Clear” or measurement key to return to the measurement screen.
8. Press the “Standardize” key and “1-Auto – enter a buffer.”
9. Follow operation manual procedure to calibrate pH probe.
10. Place at least 30 ml of the sample in a 100 ml plastic beaker.
11. Take pH probe out of 2 M KCl formaldehyde solution and rinse with deionized water using a squirt bottle.
12. Submerge the pH probe in the sample while stirring the sample using a stir bar and stir plate.
13. Press the “Measurement” key to return to the measurement screen.
14. Record the pH after it has equilibrated.

F. Data Archival and Analysis

Record sample pH if needed in its corresponding batch record and store records in a folder labeled with the run number.