

**STANDARD OPERATING PROCEDURE
FOLEY PILOT PLANT****TITLE: Media Preparation**

AUTHOR: G.W. Luli
APPROVALS: Process Change Committee

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A. Scope

Bacteria growth media components will be prepared in the Process Laboratory using the PrepTank (TS-2109) and pumped to propagation, trace metals, antifoam, sodium metabisulfite and magnesium sulfate vessels. This procedure covers the operation of the medium preparation tank used to deliver ingredients to the production area.

B. Safety and Training Requirements

Refer to UF lab safety policies regarding equipment 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 laboratory, the following safety gear will be utilized at all times:

- Lab Coat
- Safety Goggles
- Protective Gloves

C. Related Documents and SOPs

1. Experimental Plan
2. MSDS Binder
3. Steam Supply SOP-9305
4. Refrigeration System SOP-9210
5. Air System Operation SOP-9405
6. Plant pH Probe Calibration SOP-0519
7. Sampling SOP-0511
8. C5 Hydrolysate Storage SOP-2135
9. Primary Propagator 1 VS-3202A SOP-3210
10. Primary Propagator 2 VS-3202B SOP-3215
11. Trace Elements Storage SOP-3250
12. Antifoam System Operation SOP-8310
13. UV Water System Operation SOP-9555

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14. Potable Water SOP-9705

D. Preparation/Materials/Equipment

1. Antifoam

E. Detailed Procedures

a. Vessel Preparation

1. Ensure that the Steam Supply system is operational according to Steam System SOP-9305.
2. Ensure that the UV Water system is operational according to UV Water System Operation SOP-9555.
3. Ensure the Air System is operational according to Air System Operation SOP-9405.
4. Ensure that the Refrigeration System is operational according to Refrigeration System Operation SOP-9210.
5. Ensure by visual inspection that the Prep Tank TS-2109 is clean and empty before use.
6. Calibrate the instrumentation by:
 - i. pH Measurement
 - a. Calibrate pH probe AE-2109-07 according to Plant pH Probe Calibration SOP-0519.
 - b. Verify that the pH readings after calibration are the same as displayed on the HMI tag AIC-2109-07.
 - ii. Temperature Measurement
 - a. On the HMI, verify that the following temperature probes are reading current ambient conditions: TIC-2109-08 and TIC-2109-11.
7. Ensure the valves of the Media Prep Tank are positioned according to the table below.

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Line	Line Number	Valve	Position	Check
C5 Hydrolysate Pump	HYDS-2103-04-SS94	2104-V-01	Closed	
UV Water	UVW-9503-15-SS10	2104-V-04	Closed	
Aqueous Ammonia	AMX-8501-05-SS10	2104-V-06	Closed	
Tank Outlet	HYDS-2104-05-SS94	2104-V-12	Closed	
To Heat Exchanger	HYDS-2104-05-SS94	2104-V-15	Open	
		2104-V-17	Closed	
Between Heat Exchangers		2104-V-18	Closed	
		2104-V-20	Open	
		2104-V-21	Closed	
Feed Lines to Process	HYDS-2104-07-SS94	2104-V-23	Closed	
		2104-V-28	Closed	
		2104-V-29	Open	
		2104-V-30	Open	
		2104-V-31	Closed	
		2104-V-32	Closed	
		2104-V-33	Closed	
		2104-V-34	Open	
		2104-V-35	Open	
Recirculation Loop	HYDS-2104-14-SS94	2104-V-26	Open	
Low Pressure Steam Supply	SL-9302-37-CS72	2104-V-36	Open	
		2104-V-37	Open	
LP Steam to Prep Tank	SL-9302-37-CS72	2104-V-08	Closed	
		2104-V-10	Open	
LP Steam to Heat Exchanger	SL-92104-11-CS72	2104-V-11	Open	
		2104-V-19	Closed	
LP Steam to Sample Valve	SL-2104-15-CS72	2104-V-09	Closed	
Cooling Water Return	CWR-2104-12-CS51	2104-V-22	Closed	
Cooling Water Supply	CWS-9202-33-CS51	2104-V-24	Closed	
		2104-V-25	Open	
Steam to trap	CL-2104-10-CS72	2104-V-16	Closed	

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b. Media Preparation

1. Add UV water to the Media Prep Tank by:
 - a. Ensure that the bottom drain valve 2104-V-12 is Closed.
 - b. Tare weight scale to 0.0 lbs.
 - c. Open UV Water feed valve 2104-V-04.
 - d. Monitor weight scale until the desired amount of UV water has been added according to the Experimental Plan.
 - e. Close UV Water feed valve 2104-V-04.
2. On the HMI, turn on Prep Tank Agitator AG-2109 using XS-2109-03.
3. Start the recirculation loop by:
 - a. Open the tank drain valve 2104-V-12
 - b. On the HMI, start the Prep Tank Pump PT-2109 by:
 - i. On the HMI, verify the Prep Tank Pump PT-2109 in ON using XS-2109-05
 - ii. Set the speed to 100% in SIC-2109-05 with OUTPUT in MANUAL.
 - c. At the HMI, ensure the pressure in the recycle loop to 10 PSIG at PIC-2109-14
 - d. Set pressure controller PIC-2109-14 to AUTO.
4. Begin adding nutrients to the Prep Tank through the tank lid according to the Experimental Plan.
5. If the ingredients start to foam, add a small amount of antifoam using a sterile 50 mL pipette.
6. If needed, the mixture can be heated to the desired set point according to the Experimental Plan by:
 - a. On the HMI, turn off the cooling controller TIC-2109-11 by setting to Manual with an output of -5.
 - b. On the HMI, turn on the heating controller TIC-2109-08 to the desired temperature in the Experimental Plan and set to AUTO.
7. Once the mixture has achieved the temperature set points, transfer the mixture to the desired receiving vessel according to the Experimental Plan and their respective SOP.
8. Monitor the weight scale until the desired amount, or all, of the mixture has been transferred, at which point the corresponding Prep Tank transfer valve should be closed.
9. On the HMI, turn off:
 - a. The Prep Tank Agitator AG-2109 by switching XS-2109-03 to STOP
 - b. The Prep Tank Pump PG-2109 by changing output of SIC-2109-05 to -5.00.
 - c. Temperature controllers by switching TIC-2109-08 and -11 to MANUAL mode with OUTPUT of -5.

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10. At the HMI, assure the following Antifoam Transfer pumps are set to CASCADE mode.
 - a. PT-3215
 - b. PT-3214
 - c. PT-3210

F. Shut Down and Cleaning

1. Once the mixture has been transferred, rinse the tank and recirculation loop by:
 - a. Ensure that the valve used for transferring the mixture to the process tank is closed.
 - b. Spray down the Prep Tank with process water and a hose with the bottom valve open (2104-V-12) and the flexible hose disconnected from the pump.
2. Rinse the circulation loop by:
 - a. Add 20 gallons (166 pounds) of UV water.
 - b. Open the tank drain valve 2104-V-12
 - c. On the HMI, start the Prep Tank Pump PT-2109 by:
 - i. On the HMI, verify the Prep Tank Pump PT-2109 in ON using XS-2109-05
 - ii. Set the speed to 100% in SIC-2109-05 with OUTPUT in MANUAL.
 - d. At the HMI, ensure the pressure in the recycle loop to 10 PSIG at PIC-2109-14 and set pressure controller PIC-2109-14 to AUTO.
 - e. Circulate for 10 minutes.
 - f. Stop Prep Tank Pump PT-2109 by:
 - i. On the HMI, set XS-2109-05 to OFF.
 - ii. Set the output of SIC-2109-05 to -5 in MANUAL.
 - g. Disconnect the flexible hose from the suction side of the Prep Tank Pump PT-2109.
 - h. Drain the rinse water to the floor drain.
 - i. Set all valves to the initial valve setting table above.