



BIOPHARMACEUTICAL DEVELOPMENT PROGRAM

SOP Title: Operation, Cleaning and Sanitization of the Gaulin Cell Homogenizer, Model 15M-8TA
SOP Number: 12107
Revision: 04

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1. PURPOSE

This procedure describes the method for operating, cleaning, and sanitizing the Gaulin Cell Homogenizer, Model 15M-8TA at the Advanced Technical Research Facility (ATRF).

2. SCOPE

This procedure applies to Biopharmaceutical Development Program (BDP) personnel operating the Gaulin Cell Homogenizer, Model 15M-8TA to homogenize bacterial cells.

3. RESPONSIBILITIES

3.1 Director of Late Process Sciences, Biopharmaceutical Development Program (BDP)

- Defines this procedure.

3.2 Manager of Late Process Sciences, BDP

- Trains personnel on this procedure.

3.3 Manufacturing personnel

- Implements this procedure.

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- 3.4 Biopharmaceutical Quality Assurance (BQA)
- Provides quality oversight of this procedure.

4. MATERIALS AND REAGENTS

Part Number	Description	BDP Approved Substitution Permitted?
N/A	DPRO water or better	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
10117	Ethanol	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
20732	Stainless steel spatula	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO

5. EQUIPMENT

- Gaulin Model, 15M-8TA
- Recirculating water bath or chilled water loop.
- Spectrophotometer capable of reading OD₆₀₀
- Thermometer.
- Cell suspension

6. PROCEDURE

6.1 Startup of the Recirculating Water Bath

- 6.1.1 Before starting the unit, check all electrical and plumbing connections.
- 6.1.2 For the chilled water loop, connect supply and return hoses to the cooling coil.
- 6.1.3 Verify that there are no obvious leaks and that the system is in good condition.
- 6.1.4 Turn on the coolant to the cooling coil.

Confirm that the chilled water loop temperature is set appropriately (Refer to the Batch Production Record (BPR) of the Supervisor's instructions for the temperature range). Allow the coil to cool for 5 minutes minimum.

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- 6.2 Startup of the Gaulin
 - 6.2.1 Make a logbook entry per **SOP 21531** - Equipment Logs for any activity performed using the homogenizer.
 - 6.2.2 Loosen the handwheel fully by turning it counterclockwise until it stops (see Attachment 3).
 - 6.2.3 Turn the dampening needle valve clockwise until it stops, then turn it back counterclockwise 1/8 to 1/4 turn (see Attachment 1).
 - 6.2.4 Fill the bowl with Double Pass Reverse Osmosis (DPRO) water or better.
 - 6.2.5 Turn the electrical power on.
 - 6.2.6 Flush the line with 4 liters minimum DPRO water or better.
 - 6.2.7 When the water level reaches the bottom of the bowl, add 1 to 2 liters of resuspension buffer.
 - 6.2.8 When the resuspension buffer nears the bottom of the bowl, check the system integrity by applying an amount of pressure (refer to the BPR or the Supervisor's instructions for this amount). Turn the handwheel clockwise until the gauge reads the desired set range. Fine adjustment of the dampening needle valve can be made at this time such that pressure fluctuation as read from the pressure gauge is 500-2000 psig.
 - 6.2.9 Verify that no leaking is occurring around the valve body/cylinder junction (see Attachments 2 and 3).
 - 6.2.10 Add the cell suspension to the bowl. Retain a 1 mL sample of the cell suspension for homogenizing efficiency determination.
 - 6.2.11 Collect the cell lysate in a container.
 - 6.2.12 Monitor the lysate temperature and record on the BPR if required.
 - 6.2.13 Retain a 1mL sample of the first pass lysate for determination of homogenizing efficiency.
 - 6.2.14 As the last of the cell suspension is going into the Gaulin, pour 500 to 1000mL of suspension buffer.

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- 6.2.15 When the suspension buffer is down to the bottom of the bowl, the First Pass lysate can be added back to the bowl for a second pass.
 - 6.2.16 Continue processing until the remainder of the First Pass lysate is processed through the Gaulin a second time.
 - 6.2.17 Retain a 1 mL sample of the second pass lysate for determination of homogenizing efficiency.
 - 6.2.18 Repeat steps 6.2.13 to 6.2.16 as often as necessary to execute the desired number of passes through the homogenizer (refer to the BPR or the Supervisor's instructions).
 - 6.2.19 Relieve the system pressure by turning the handwheel counterclockwise until it stops. If the gauge still shows pressure, turn the needle valve counterclockwise slowly until the gauge pressure starts to drop.
 - 6.2.20 Return the dampening needle valve to its original 1/8 to 1/4 turn setting.
 - 6.2.21 With no pressure, flush the fluid path with suspension buffer.
 - 6.2.22 Per the Supervisor's instructions, determine homogenizing efficiency by the absorbance reading OD600 of the lysate for each pass through the Homogenizer. Generally the homogenizing process is finished when the absorbance reading OD600 becomes asymptotic. Process could be stopped earlier per Supervisor instructions or Master Production Record (MPR's) requirements. All dilutions are made in the cell suspension buffer.
 - 6.2.23 Turn the coolant off and proceed to system cleaning and sanitization
- NOTE:** All attachment references require Attachment 4 to properly identify each component description.

7. PREVENTATIVE MAINTENANCE

- 7.1 If the machine appears to be operating inefficiently, the high wear components may need to be replaced. See the Gaulin manual for high wear items and periodic maintenance schedules.
- 7.2 Make a logbook entry reflecting any maintenance done to the homogenizer include date, time and description of maintenance per **SOP 21531** - Equipment Logs.

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8. CLEANING

- 8.1 Rinse the homogenizer three times by filling the feed vessel completely with DPRO water or better, then running the entire volume of water through the homogenizer to a waste container.
- 8.2 Rinse the homogenizer with 0.5M NaOH twice as described in 6.1, substituting 0.5M NaOH for water.
- 8.3 Fill the feed vessel completely with 0.5M NaOH and run through the homogenizer until feed vessels is 1/3-2/3 full, then turn off the homogenizer, close the discharge tubing and allow the homogenizer to sit for a minimum of one hour.
- 8.4 Run the remainder of the 0.5M NaOH solution in the feed vessel though the homogenizer to a waste container.
- 8.5 Rinse the homogenizer with DPRO or better water as described in 6.1 until conductivity is < 5 μ S/cm, then allow the homogenizer to run until no more liquid is being discharged, then turn it off. During this step, collect rinse samples per SOP 12169 - Rinse Water Sampling for Production Equipment, and submit for analysis of cleaning. If it is interproduct cleaned, follow guidelines in **SOP 21529 - Equipment Interproduct Cleaning and Clearance.**
- 8.6 Add 100% EtOH until the feed tank has been filled.
- 8.7 Turn on the power and allow the homogenizer to run the entire volume of EtOH to a waste container.
- 8.8 Turn off the power.
- 8.9 Turn the needle valve clockwise until it stops.
- 8.10 Cover the feed vessel.
- 8.11 Dispose of waste in accordance with established Environmental Health and Safety (EHS) guidelines.
- 8.12 Document the date, time, NaOH lot number and expiration date, NaOH contact time, EtOH lot number and expiration date, and product/lot number processed in the homogenizer on **Form 12149-01** (see **SOP 12149 - General Cleaning of Process Equipment**).
- 8.13 Make a cleaning entry in the logbook per **SOP 21531 - Equipment Logs.**

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9. DOCUMENTATION AND RECORDS

- 9.1 Document this procedure in the BPR (when applicable) and on **Form 12149-01**.
- 9.2 Document use and cleaning in the equipment logbook. Entry should include production lot number, date and initials.

10. REFERENCES AND RELATED DOCUMENTS

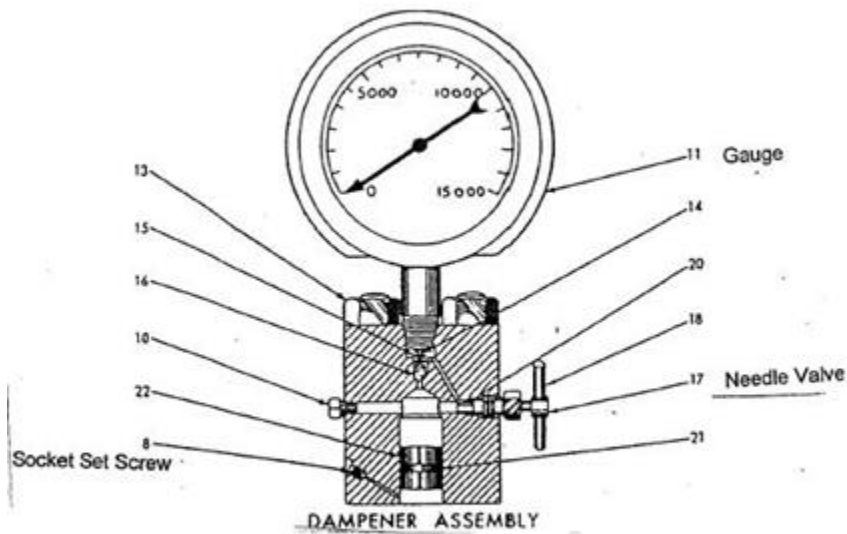
Document Number	Title
N/A	Gaulin Corporation Laboratory Model 15M Homogenizer Manual.
12149	General Cleaning of Process Equipment
12169	Rinse Water Sampling for Production Equipment
21529	Equipment Interproduct Cleaning and Clearance
21531	Equipment Logs

11. ATTACHMENTS

- Attachment 1 Dampener Assembly
- Attachment 2 Laboratory Homogenizer, SMD, or SP Pump
- Attachment 3 Single Stage Homogenizer or SMD Assembly
- Attachment 4 Parts List

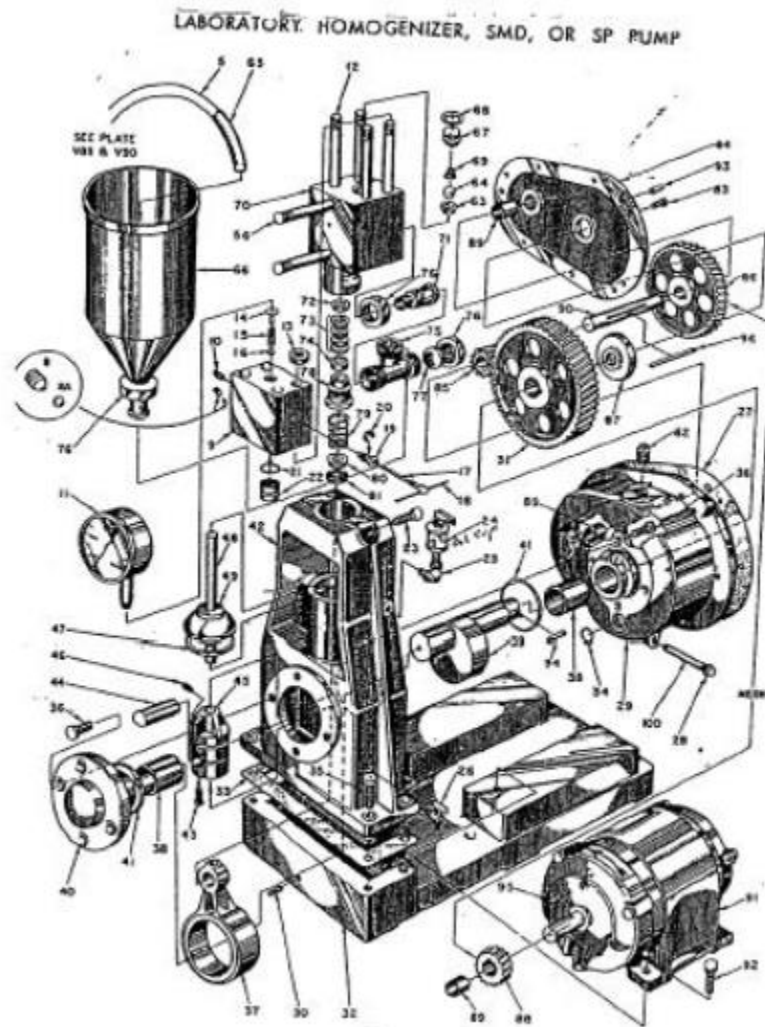
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Attachment 1 Dampener Assembly



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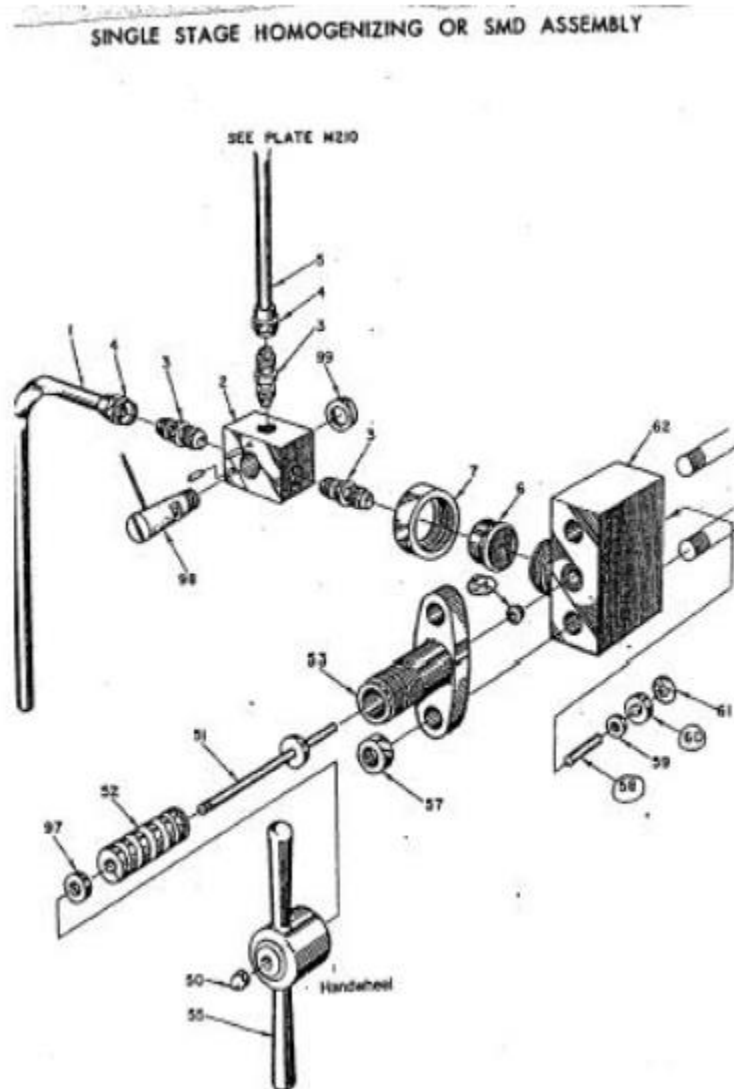
Attachment 2 Laboratory Homogenizer, SMD, or SP Pump



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Attachment 3 Single Stage Homogenizer or SMD Assembly



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Attachment 4 Parts List

PARTS LIST

ITEM	QTY.	DESCRIPTION	ITEM	QTY.	DESCRIPTION
1	1	Cylinder Block	44	1	Three-Way Valve Handle
2	2	Upper Cap Stud Nut	45	1	Three-Way Valve Nut
3	1	Upper Cap	46	1	Inlet Connection Nipple
4	1	Upper Cap Plug	47	1	Inlet Connection Nut
5	1	Upper Cap Plug Gasket	48	1	Tank Elbow
6	2	Upper Cap Stud	49	1	Tank Nut
7	1	Pump Valve Seat	50	1	Tank
8	1	Pump Ball Valve	51	1	Discharge Tube
9	1	Pump Ball Valve Spring	52	1	Discharge Tube Nut
10	1	Plunger	53	1	Bypass Tube
11	1	Plunger Ring	54	1	Bypass Tube Nut
12	4	Plunger Packing	55	1	Valve Body (First-Stage)
13	2	Packing Adjusting Ring	56	1	Valve Rod Washer (First-Stage)
14	1	Packing Adjusting Screw	57	1	Homogenizing Valve Spring (First-Stage)
15	1	Crosshead Cover Spring	58	1	Valve Rod (First-Stage)
16	4	Crosshead Cover Packing Spring	59	1	Valve Body Stud Nut (First-Stage)
17	1	Crosshead Cover Packing	60	1	Valve Rod Packing (First-Stage)
18	1	Crosshead Cover	61	1	Homogenizing Valve (First-Stage)
19	2	Crosshead Cover O-Ring	62	1	Impact Ring (First-Stage)
20	1	Cylinder Set Screw	63	1	Homogenizing Valve Seat (First-Stage)
21	1	Well Drain	64	1	Homogenizing Valve Seat Gasket (First-Stage)
22	1	Oil Filler/Breather Cap	65	1	Handwheel (Second-Stage)
23	1	Draincock	66	1	Valve Rod Washer (Second-Stage)
24	1	Oil Sight Glass	67	1	Homogenizing Valve Spring (Second-Stage)
25	1	Water Drip Tube Assembly	68	1	Valve Rod (Second-Stage)
26	1	Handwheel (First-Stage)	69	2	Valve Body Stud Nuts (Second-Stage)
27	1	Handwheel Support (First-Stage)	70	1	Valve Rod Packing (Second-Stage)
28	2	Valve Body Stud (First-Stage)	71	1	Homogenizing Valve (Second-Stage)
29	2	Valve Body Stud Nut (First-Stage)	72	1	Valve Seat (Second-Stage)
30	1	Dampener Body	73	1	Valve Seat Gasket (Second-Stage)
31	4	Dampener Body Stud Nuts	74	1	Valve Body Stud (Second-Stage)
32	1	Dampener Knob	75	1	Valve Body (Second-Stage)
33	1	Dampener Gauge	76	1	Handwheel Support (Second-Stage)
34	1	Dampener Check Valve Ball	77	1	Motor
35	1	Dampener Check Valve Spring			
36	1	Dampener Needle Valve			
37	1	Dampener O-Ring			
38	1	Dampener Back-Up Ring			
39	1	Dampener Support Ring			
40	3	Dampener Pipe Plug			
41	1	Dampener Plunger			
42	1	Gasket — Dampener to Cylinder			
43	1	Three-Way Valve Bypass			