



Bioreactor SOP for HEK 293 Cells

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ABSTRACT

The combination of mini-Bioreactors from Applikon and their myControl system, allows for flexibility and tracking of growth conditions for controlled cell culture. The inputs include pH, dissolved oxygen (DO), temperature, agitation, foam, and level with PID control. This small-scale, stirred-tank bioreactor culture is a cost-effective method to develop new protocols and to scale up from petri dish cultures. ¹

INTRODUCTION

The following SOP was developed for the batch suspension culture of HEK293 F cells from Invitrogen. It can be used as a basis for the development of cell type-specific SOPs using pluripotent stem cells and their differentiated progeny. The bioreactor system is the Applikon miniBio coupled with the myControl controller.

MATERIALS

CELL LINES AND REAGENTS

- 1) HEK 293 F Cells (Cat# 11625-019, Invitrogen)
- 2) CD 293 (1X) (Cat# 11913-019, Life Technologies)
- 3) L-Glutamine (Cat# 25030-164, Fisher Scientific)
- 4) Primocin (Cat# NC9392943, Fisher Scientific)
- 5) TRUEResult Blood Glucose Starter Kit (Cat# B002CGT08S, Amazon)
- 6) TRUEtest Test Strips, 100 count (Cat# B002CGT09M, Amazon)
- 7) Catalyst Glucose (GLU) Slide (Cat# 98-11076-01, IDEXX)
- 8) Catalyst Sample Cups (Cat#98-12128-00, IDEXX)
- 9) Sigmacote (Cat # SL2 100ml, Sigma)
- 10) Pluronic F68 (Cat# 24040-032, Life Technologies)
- 11) Antifoam C (Cat# A8011-250ML, Sigma Aldrich)
- 12) 7X Cleaning Solution (Cat#76-670=93, MP Biomedicals)
- 13) Distilled Water

EQUIPMENT

¹ Applikon-biotechnology.us

(All items are **CRITICAL** to the proper setup and function of the miniBioreactors)

- 1) PC
- 2) *myControl* controller
- 3) Heating blanket (Cat #Z311021025)
- 4) MiniBio Reactor-250ml (Cat #Z611025010)
- 5) Bioreactor Assembly-250ml (Cat #V3LP070011)
- 6) Stand for 250ml MiniBio Reactor (Cat #V3LP070021)
- 7) Vessel Dished Bottom 250 ml (Cat #V3MP072501)
- 8) Bottom Flange 250 ml Reactor (Cat #V3MP070051)
- 9) Top Plate 250 ml Reactor (Cat #V3MP070011)
- 10) Silicone Gasket 250 ml Reactor (Cat #V1S4TC0044)
- 11) Silicone O-ring ID 66.34 x 2.62 (Cat #V1S4ARP146)
- 12) Silicone O-ring ID 56.82 x 2.62 (Cat #V1S4ARP140)
- 13) Reactor Clamp 250 ml Reactor (Cat #V3MP071201)
- 14) Liquid Addition/Sample Bottle (Cat #Z812070251)
- 15) Stepper Stirrer Motor for MiniBio (Cat #Z510002110)
- 16) Stirrer Assembly Lipseal MiniBio 250 ml (Cat #Z813150251)
- 17) Lipseal Stirrer (Cat #V3LP070161)
- 18) Silicone O-Ring ID 2.20x1.60 (Cat #V1S4022X16)
- 19) Stirrer Shaft for 250 ml MiniBio (Cat #V3MP070752)
- 20) Marine Impeller (Cat# Z813140251/ Cat# Z813140501)
- 21) Septum Assembly MiniBio (Z813020251)
- 22) Septum (Cat #V0W5024002)
- 23) Clamping Nut Universal M8x1 (Cat #V3MP100171)
- 24) Clamping Nut Sample Pipe M8x1 ID3.2 (Cat # V3MP100121)
- 25) Clamping Nut Universal M8x1 (Cat # V3MP100171)
- 26) Sensor Holder pH/DO MiniBio (Cat # Z813000251)
- 27) Blind Nut M8x1 (Cat # Z813220251)
- 28) Blind Nut M12x1 (Cat # Z813220252)
- 29) Tool for Clamping Nut D8 / D12 part of the Startup Kit (Cat # V3MP079121)
- 30) Tool for Clamping Nut D9 part of the Micro Valve Assembly (Cat # V3MP079131)
- 31) S/A Bottle Cap Connector M6 (hb 2.5 mm) (Cat # V3MP070821)
- 32) Seal for Cap 15 ml Tube (Cat # V3MP070811)
- 33) Cap for 15 ml Tube (Cat #V3MP070801)

- 34) Conical Centrifuge Tube 15 ml PP (100 pcs) (Cat #Z850101510)
- 35) Tube for Cap Reagent Bottle L=419 (Cat # V0W2130084)
- 36) PharMed® Ismaprene Extension Tubing, 3 m, Wall Thickness ~0.86 mm 1.02 mm ID (Cat#SC0343, IDEX Health and Science)
- 37) Thermo Scientific™ Nalgene™ Pharma-Grade Platinum-Cured Silicone Tubing 1/16x 1/8 x 1/32 (Cat# 14-179-110)
- 38) Air Filter 0.2µm PP/PTFE Luer L. M + Luer Slip (Cat # V0W1040020)
- 39) Male Luer – Male Luer Connector PC (Cat # V0W0030116)
- 40) Sample System Single Use for MiniBio Reactors (Cat # Z850000251)
- 41) Syringe 10ml with Luer Lock (Cat # V0W6500002)
- 42) Syringe with Luer lock tip 3ml, case of 800 (Cat# BD309657, Bioexpress)
- 43) Adapter 2x Female Luer Lock (Cat #V0W0030121)
- 44) Air Filter 0.2µm (Cat # V0W1040020)
- 45) Syringe filter, hydrophobic (Cat# 16596-HYK, Sartorius Stedim Biotech)
- 46) Aervent®-50 Filter Unit 0.2 µm hydrophobic 1/8 in. HB/HB (Cat# MTGR05010, Millipore)
- 47) SLFG025LS | Millex-FG Filter Unit, 0.2 µm, hydrophobic PTFE, 25 mm, PVC, ethylene oxide sterilized (Cat# SLFG025LS, Millipore)
- 48) Straight Through Reduction Tube Fitting with 500 Series Barbs, 1/8" (3.2 mm) and 1/16" (1.6 mm) ID Tubing, Animal-free Natural Polypropylene (Cat# N013/004-6005, Value Plastics)
- 49) Male Luer Connector 2x (Cat # V0W0030116)
- 50) Male Cap (Cat #V0W0030113)
- 51) Swabable Valve (Female Luer Lock) (Cat # V0W0030103)
- 52) Y-Piece, 2xFemale Luer Lock, 1xMale (Cat # V0W0030104)
- 53) Hose Barb 1/8" – Female Luer Lock (Cat # V0W0030109)
- 54) Cable Tie Black (Cat # V0R1451030)
- 55) Silicone Tubing Size 14 (Cat # V0M5219030)
- 56) pH Sensor for MiniBio 250 ml (Cat #Z001014070)
- 57) Sensor Cable pH 65 cm (Cat #Z100065010)
- 58) O-ring Silicone 6.02 x 2.62 (Cat #V1S4ARP108)
- 59) O-ring Viton 6.02 x 2.62 (Cat #V1S6ARP108)
- 60) DO Sensor for MiniBio 250 ml (Cat #Z010014070)
- 61) Sensor Cable DO 65 cm (Cat #Z100065012)
- 62) O-ring Silicone 6.02 x 2.62 (Cat #V1S4ARP108)
- 63) O-ring Viton 6.02 x 2.62 (Cat # V1S6ARP108)

- 64) Low Drift Membrane Module Kit (Cat #Z110000470)
- 65) Clamping nut Universal M8x1 (Cat #V3MP100171)
- 66) O-ring 2.90x1.78 (Cat #V1S4ARP006)
- 67) Temperature Sensor Pt-100 (Cat #Z034014010)
- 68) Autoclave Support 4x MiniBio 250 & 500 ml Assembly (Cat # Z811110251)
- 69) Startup Kit for the MiniBio Reactor (Cat # Z811000251)
- 70) Nalgene Pharma Grade Silicone Tubing (Cat# 8600-0020, Thermo Scientific)
- 71) Peroxide-Cured Silicone Tubing (Cat#96400-16, Cole Parmer)
- 72) Male Luer (Cat#45513-00, Cole Parmer)
- 73) Female Luer (Cat#45512-00, Cole Parmer)
- 74) 4" Miniature Cable ties (Cat#AL-04-18-9-C, Advanced Cable Ties, Inc.)
- 75) AdvantaFlex TRE tubing (Cat# AF7500077, Value Plastic)
- 76) Male Luer (Cat#N013/004-6005, Value Plastics)
- 77) Male Luer (Cat#MTLL013-6005, Value Plastics)
- 78) Female Luer (Cat#FTLL013-6005, Value Plastics)
- 79) Y-fitting (Cat#Y230-6005, Value Plastics)
- 80) ¼ Tube clamps (Cat#4VYC6, Grainger)
- 81) Needlefree Swabable Female Luer (Cat# NVFMLLPC, Value Plastics)
- 82) Peltier Cooler (Cat#1335, Adafruit)
- 83) Power Supply for Peltier cooler (Cat#352, Adafruit)
- 84) Power adapter for Peltier cooler (Cat#368, Adafruit)

PROCEDURE

A. RINSING

- 1) Rinse the Vessel, Impeller, Stirrer Shaft, Top Plate, Silicone Gasket and Silicone O-ring with DI water.
- 2) Allow parts to air dry.

B. SIGMACOTE APPLICATION

Sigmacote is a siliconizing agent that forms a covalent, microscopically thin film on glass.

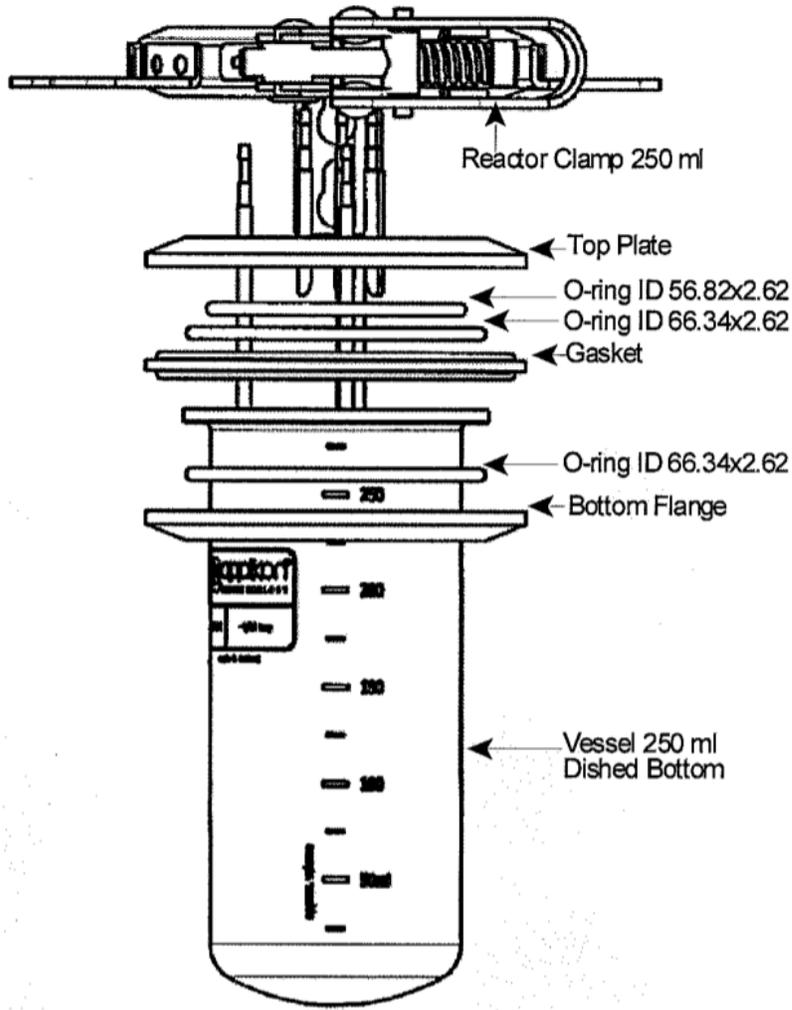
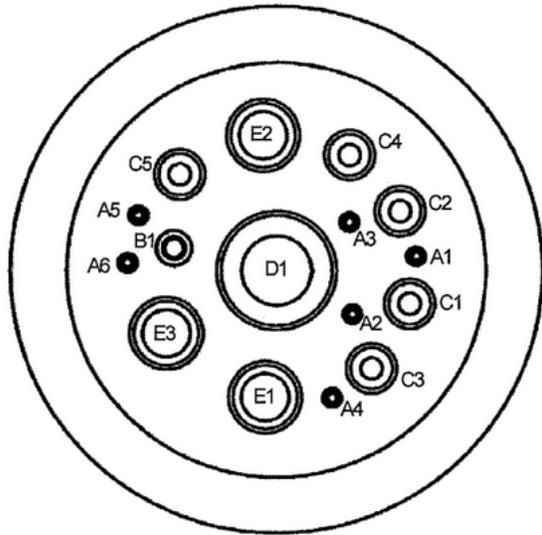
- 1) This needs to be done once every 10 runs.
- 2) **This should be done in a chemical hood.**
- 3) Add 10ml of Sigmacote to glass vessel and rotate to allow for all surfaces to be coated with Sigmacote.

- 4) Place the impeller into the Vessel and gently rotate to coat the impeller with Sigmacote.
- 5) Pour the 10ml of Sigmacote from the vessel into a 15ml conical and put the Stirrer Shaft into the 15ml conical so as to coat that with the Sigmacote.
- 6) **Caution: Do not let the O-ring get in contact with the Sigmacote.**
- 7) Since the Sigmacote is reusable, it can be poured back into the stock bottle.
- 8) Allow all the parts to air dry.

C. BIOREACTOR ASSEMBLY

Refer to Table and Diagram

Port #	Size	Description
A1	2.6mm Hose Barb	Sample port
A2	2.6mm Hose Barb	Gas Overlay
A3	2.6mm Hose Barb	Base Addition
A4	2.6mm Hose Barb	Gas Sparger Pipe
A5	2.6mm Hose Barb	Inoculation
A6	2.6mm Hose Barb	Drain
B1	4mm pocket, sealed	Temperature Pocket
C1	M8x1	M8x1 Plug nut
C2	M8x1	Level Sensor (M8x1 to 3.2mm)
C3	M8x1	M8x1 Plug nut (Septum)
C4	M8x1	M8x1 Plug nut
C5	M8x1	M8x1 Plug nut
D1	M20x1	Stirrer Port
E1	M12x1	DO Sensor (M12x1 to 6.4mm Clamping nut)
E2	M12x1	pH Sensor (M12x1 to 6.4mm or 8.0mm Clamping nut)
E3	M12x1	Condenser Port (M12x1 to 6.0mm Hose Barb)



Ensure O-rings are present and intact for all ports when threading parts onto the headplate. Inspect all large O-rings on the head plate assembly before assembling. All tubing is peroxidized cured silicone tubing or equivalent, except the tubing that goes into the peristaltic pump head, this is Ismaprene tubing, with superior tensile strength. Secure all tubing connections with an appropriately sized zip tie.

1. Install Stirrer in port D1.
2. Install M8x1 plug nuts in ports C1, C3, C4, and C5.
3. Install level sensor in port C2 to desired height.
4. Install 15" of (1/8") 3.2mm ID tubing (Cat# 14-179-110) on condenser port (E3). Attach hydrophobic exhaust filter (Cat# MTGR05010) to end of tubing (inlet side connected to tubing).
5. Install pH sensor in port E2. Calibrate before autoclaving (see calibration section below). There are two different pH probe sizes, 6.4mm (old) and 8mm (new) diameter. Use the appropriate sized clamp nut.
6. Install DO sensor in port E1. **Calibrate after autoclaving.**
7. Port A1: This port is used for sampling. Install short length of (1/16") 1.6mm ID tubing (Cat# N013/004-6005). Install a 1/4" tube clamp. Then install a Y connector to which a short length of (1/16") 1.6mm ID tubing is attached on either side. Install a 1/4" tube clamp on both tubes. On one end attach a 1/16" hose barb to female luer connector on the tubing. Then install a sampling connector (Cat#NVFMLLPC Value Plastics). On the other end, attach a syringe filter (Cat# 16596-HYK).

NOTE: You want to minimize the length of tubing used in order to minimize the dead volume in the sampling tube.

8. Ports A2 and A4: These ports are used for the gas supply. A4 is the sparging line and A2 is the overlay line. Both ports get a clamp and are connected together via a Y. Both ports get the same gas supply. The on/off position of the clamps controls where gas is flowing. Install ~20" of 1/16" ID tubing (Cat# N013/004-6005) on ports A2 and A4. Install 1/4" hose clamp on both tubes. Join both tubes with a 1/16" Y connector. Install 1/16" ID tubing on the Y connector. Install a 1/16" hose barb to 1/8" hosebarb connector. Install 1/8" ID tubing (Cat# 14-179-110) (long enough to reach the controller). Install a 1/8" hosebarb to female luer. Install a hydrophobic filter (Cat# SLFG025LS) with male and female luer ports.
9. Port A3: Install a long 1/16" ID tubing (Cat# SC0343) with 1/4" clamp onto port A3. Ensure the tubing is long enough to thread through the base addition pump on the controller. Install a 1/4" clamp. Connect tubing to steel cap and liquid addition conical centrifuge (15ml) tube. Add hydrophobic filter onto female luer port on steel cap. When installing tubing into peristaltic pump, be careful not to cut and puncture tube on the tubing clamp of the pump.
10. Port A5: Inoculation port. Attach a 1/16" (Cat# N013/004-6005) tubing with clamp. Add a 1/16" to 1/8" connector. Add a 6" length of 1/8" tubing. Add a 1/8" hosebarb to female luer connector.

11. Close all unused ports.

D. EXHAUST TUBING

- Install the Peltier cooler by attaching it onto the frame of the bioreactor above the headplate. Coil the long exhaust tubing on the cooling surface to maximize contact between the exhaust tubing and cooling surface. Zip tie the tubing in place, making sure that the tubing is not clamped by the tension of the tie. Plug the cooler into the power supply to turn on the cooling unit. Over time, the tubing will become encapsulated in solid ice. Water in the exhaust gas will condense out.

E. INOCULATION BOTTLE ASSEMBLY

1. Build the inoculation bottle according to the picture above. Tubing is 1/8" (3.2mm) ID tubing.
2. Tighten cap and cover exhaust port and inoculation port with autoclave paper.



F. CALIBRATIONS:

- Open both Luke and Leia in two different tabs on the PC if both are being used or open whichever one is being used.
 - Log in as system engineer from the Home Page: Password-0000
1. pH
 - **Caution: always keep pH sensor in 3M KCl or temporarily in water.**
 - Select the pH sensor tab and press the methods tab.
 - Immerse the pH sensor in the buffer solution (e.g. pH 7) and press start for the 2-point calibration option.
 - Press the data field called Value 1 and insert the pH value of the first buffer and then proceed to enter the temperature of that buffer.
 - Press Start.
 - On completion rinse the sensor and wipe dry.
 - Immerse in the second buffer solutions (e.g. pH 4)

- Press the data field called Value 2 and insert the pH value of the second buffer. Also insert the temperature of this buffer.
- Press Start.
- On completion, evaluate and accept the calibration values and close the presented message window.
- Click the home screen.

2. Dissolved Oxygen (DO)

- **CAUTION: This calibration is performed after the autoclave process is completed. (See section G)**
- Select the DO sensor tab and select the Settings tab to select air as the calibration gas.
- Press the methods tab and hit Begin for the 2-point calibration option.
- Aerate the bioreactor with air for an interval of 15 minutes. This should be done by sparging. (If the reading stabilizes prior to 15 minutes, proceed to the next step).
- Press the data field called Value 1 and enter the saturation value of 100%. Wait till the reading is stable.
- When stable hit Start to accept the calibration point.
- After confirming that the reading is stable on the myControl, proceed to the second point calibration.
- Aerate the bioreactor with nitrogen for an interval of 15 minutes. This should be done by sparging. (If the reading stabilizes prior to 15 minutes, proceed to the next step).
- Press the data field called Value 2 and enter the saturation value of 0%. Wait till the reading is stable.
- When stable hit Start to accept the calibration point.
- On completion, evaluate and accept the calibration values and close the presented message window.
- **Recalibrate after 6 hours.**
- **Inoculate cells after this second calibration is completed.**
- Turn nitrogen on and test of the DO drops as expected.

3. Temperature

- 1-point calibration done once a year and 2-point calibration done once in a lifetime.
- Select the Temperature sensor tab and press the methods tab.
- Prepare a beaker with 100ml of water and sufficient ice cubes. Stir gently for approximately 5 minutes with a magnetic stirrer.
- Hit Start at the 2-point calibration option.

- Press the data field called Value 1 and enter 0°C. Immerse the temperature sensor into the ice bath and when the value stabilizes press Start.
- When completed a second calibration point is requested. Prepare a beaker with boiling water and insert the temperature sensor into this beaker while stirring with a magnetic stirrer.
- Press the data field called Value 2 and enter 100°C.
- Press Start. When completed accept and close the message window.

G. AUTOCLAVE:

1. Cover all open ends and filter ends with autoclave paper and autoclave tape.
2. Ensure all connections are secure and the bioreactor is sealed, except for the exhaust tube clamp, which should be left open to prevent pressure build up.
3. Autoclave on 25 minute liquid cycle.

H. BASE PREPERATION:

1. Prepare 9% sodium bicarbonate solution (9% w/v). Heat the solution to help dissolve solid. Filter this solution.
2. After autoclaving, bring the setup into a biosafety cabinet.
3. Fill the 15ml conical tube with 10-15ml of saturated sodium bicarbonate solution.
4. Tighten the stainless steel base cap adaptor.
5. When installing tubing into peristaltic pump, be careful not to cut and puncture tube on the tubing clamp of the pump.

I. MEDIA PREPARATION

1. HEK 293 media consists of CD293, 4mM L-glutamine, Primocin for antibiotic resistance (1:500X).
2. Pluronic is added to this media recipe at 0.4% by volume. This was added to reduce hydrodynamic damage to the cells.
3. Antifoam C is also added to the media recipe at 0.015% by volume to reduce the foam created by stirring.

J. INOCULATION:

1. After autoclaving, let the inoculation bottle cool. The inoculum can then be transferred to the inoculation bottle.
2. The inoculum consists of the media and cells at concentration of 300,000 cells/ml.
3. The male luer from the bottle attaches to the female luer of the bioreactor.

4. The bottle is inverted and the inoculum feeds into the bioreactor by gravity. The displaced air is vented out from the vent line through the filter.

K. DATA COLLECTION:

1. Data from the Controller is collected from the BioXpert software.
2. Collected parameters are: time, pH, temperature, dO₂%, level sensor, stir rate, and total air volume.
3. At the start of the run, connect to the bioreactor and record.

NOTE: File size limits either the collection interval or the duration of the run. The shortest interval is 1 minute.

L. SAMPLING:

1. Done at regular, consistent intervals – every 12 hours or every 24 hours to be able to measure proliferation and viability.
2. Use a 3ml syringe to collect 2 ml of the culture to do a cell count and check for viability.

CAUTION: extra volume is removed to adjust for any dead volume from previous samplings.

3. Inject 5-7 ml of air using the large syringe on the other end of the y-clip to flush back media from the port into the vessel.

CAUTION: If this is not done, in addition to keeping very short tubing, the dead volume will increase extensively and results will be skewed.

4. Use the Countess II or hemocytometer for cell count.
5. Glucose measurements can be done with the glucose monitor and test strips. A sample of fresh media should be measured to normalize the sampled cell culture media against. For greater sensitivity the IDEXX Catalyst Dx Chemistry Analyzer can be used that can detect and analyze the glucose concentration (in addition to other metabolites) from a 300µl of the fluidics sample.
6. Record cell count, viability, and normalized glucose concentration.

M. TERMINATION, DISSASSEMBLY AND CLEANING:

1. Turn off all circuits and loops on the PC.
2. Save all the data.
3. Detach all tubes/wires that connect the bioreactor to the myControl controller and turn off the controller from the back.
4. Switch off all gases at the source.
5. Discard all the media and rinse with distilled water.
6. Add 10ml of the 7X cleaning solution to a liter of DI water and add to the bioreactor vessel.

7. Close the head plate and put onto stand
8. Let this sit over night to soak the components in this solution.
9. Rinse again the next day the whole setup well with distilled water and then once more with double distilled water (Millipore water).
10. Discard all tubing, except Ismaprene tubing, which can be reused. Only keep the luer locks and clamps for a few runs.

TIMING

1. Steps A+B, 15 minutes
2. Steps C+D+E, 2 hours
3. Step F1, 30 minutes
4. Step F2, 8 hours (since calibrations has to be done 2x at an interval of 6 hours)
5. Step F3, 30 minutes
6. Step G, 1 hour (includes cooling time post-autoclaving)
7. Step J, 15 minutes
8. Step L, 20 minutes
9. Step M, 1 hour