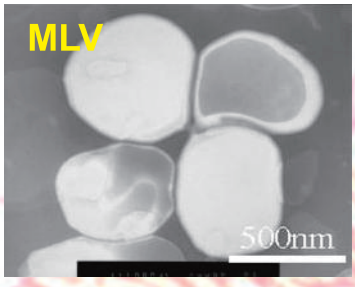
L i p o s o m e A u t o M a k e r

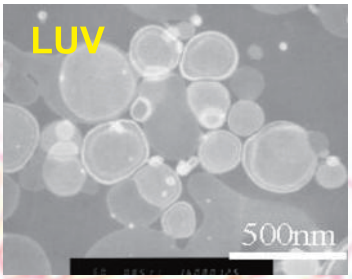
Automated Multi-functional Liposome Manufacturing Equipment

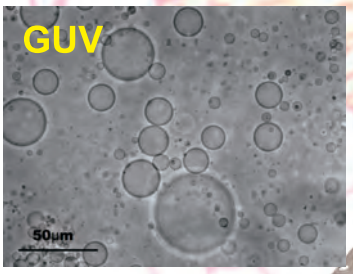
plus Programmable Bioreactor

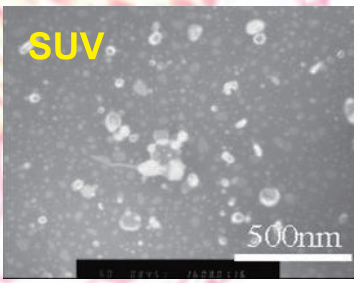
Partnership Project Approved by the Ministry of Economy, Trade and Industry “New Coordination Support Project” on June 26, 2009

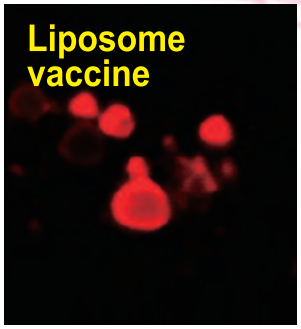
One-touch operation, produces any type of liposomes automatically and repeatedly











Reference: Liposome Engineering Laboratory,Inc.
Hashimoto Electronic Industry Co.,Ltd.

Features and benefits of Automated Multi-functional Liposome Manufacturing Equipment

1. This is the only unique piece of equipment in the world that can prepare, with only vortex-mixing, not only MLV (multilamellar vesicles) but also LUV (large unilamellar vesicles) and GUV (giant unilamellar vesicles). SUV can be made automatically additional Sonication-Units.
2. With our development of an automated extruder that will be attached to this equipment, uniform LUV (for example, 100 nm size) will be able to be prepared and available for use in DDS (drug delivery system). This will result in easy preparation and development of liposomal pharmaceuticals for cancer therapy (cancer cells usually create angiogenic vessels with intercellular spaces around 100 nm between their epidermal cells).
3. This equipment can prepare large amounts of GUV, suggesting that this equipment is useful for developing artificial cells and the next generation types of liposome encapsulating viruses or micelles. In addition,, the prepared GUV can encapsulate very high amounts of low molecular drugs and siRNA as well as intact proteins such as antibody, enzyme, etc, which can also be used for DDS by reducing their size.

Liposomes encapsulating pharmaceuticals, genes, nucleic acids, antibodies, enzymes, etc.

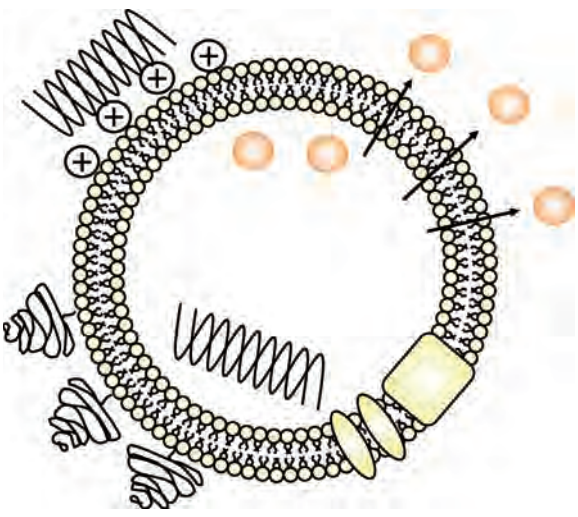
Liposomes encapsulating pharmaceuticals, genes, nucleic acids, antibodies, enzymes, etc.

Compound	Liposome	Concentration (mM)	Trapping efficiency (%)
Calcein	GUV	50	60
	GUV→LUV	50	12
	LUV	50	12
γ-Globulin	GUV	50	50
	GUV→LUV	50	24
Rh-dextran	GUV	50	32
	GUV→LUV	50	18
	LUV	50	17

This equipment reduces the time necessary for one cycle of preparation, makes multiple overnight-productions and has excellent reproducibility. Thus unskilled people can operate it and prepare liposomes easily although manual preparation is quite difficult.

Functional Liposomes that can be Produced with the Automated Liposome Manufacturing Equipment

1. Liposomes
   Multilamellar vesicles (MLV)
   Small unilamellar vesicles (SUV)
   Large unilamellar vesicles (LUV)
   Giant unilamellar vesicles (GUV)
2. Liposomes encapsulating pharmaceuticals, genes, nucleic acids, antibodies, enzymes, etc.
3. Liposomes sensitive to temperature, pH, magnetism, ultrasound, etc.
4. Liposomes coated with PEG, saccharide chains, etc.
5. Liposomes with bound ligands, such as proteins, peptides, nucleic acids
6. Reconstituted liposomes, such as proteoliposomes and virosomes
7. Liposome vaccines



Overview of Liposome Preparation Procedure

Dissolve phospholipid in ether (lipid ether)

Load the equipment with pertinent solutions

Enter the amount and conditions of preparation for the liposome

Press the preparation start icon on the touch panel

Automated preparation start

Dispense the lipid ether into a test tube

Dispense the lipid ether and GUV-specific reagents into a test tube

Make a lipid film on the wall of the test tube by vortexing under the appropriate temperature and reduced pressure

Inject the buffer into the test tube, and dissolve the lipid film by vortexing under the appropriate temperature

MLV is completed

Transfer the MLV to the ultrasonic homogenizer, and then apply ultrasonic vibration

SUV is completed

Inject ether into the test tube, and dissolve the lipid film by vortexing under the appropriate temperature

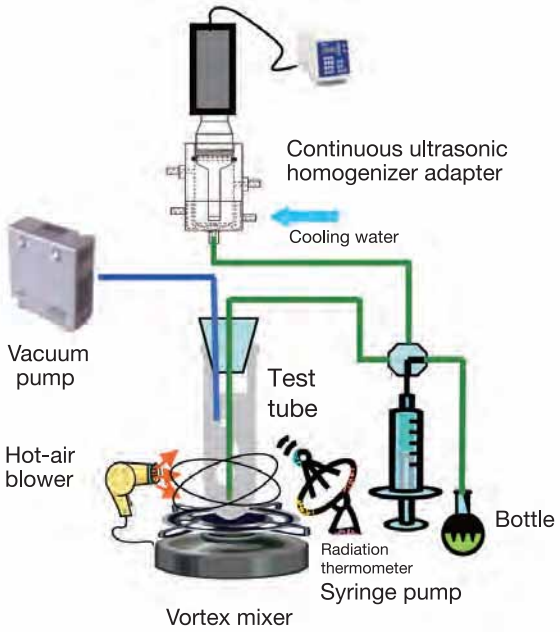
Inject the buffer into the test tube, and carry out vortexing under the appropriate temperature and reduced pressure

LUV is completed

Inject the buffer into the test tube, and carry out vortexing lightly under the appropriate temperature

GUV is completed

Working Model of Automated Multi-functional Liposome Manufacturing Equipment



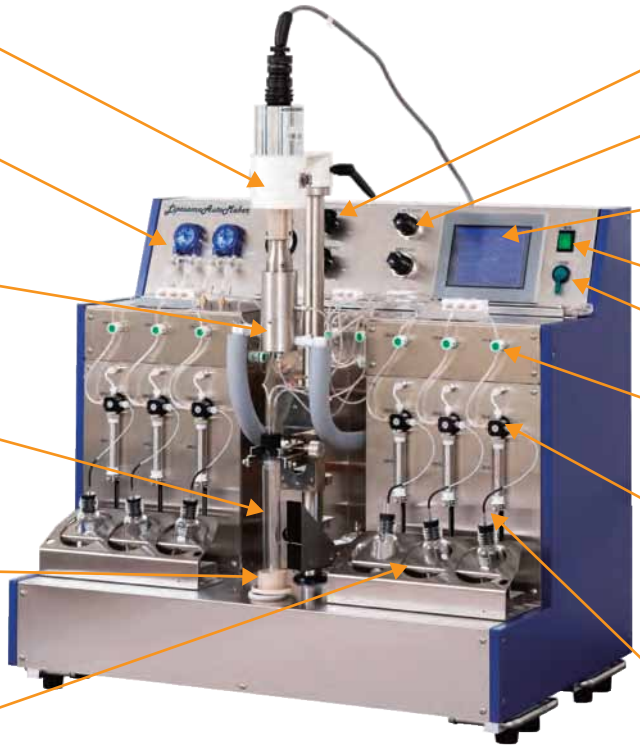
- Liposome vaccines encapsulating soluble antigens (MLV, SUV, and LUV): Inject aqueous solution-based soluble antigen.
- Liposome vaccines encapsulating lipid-soluble antigens (MLV, SUV, and LUV): Inject organic solvent-based lipid-soluble antigen.
- Reconstituted liposomes: Pump to the test tube the liposome solution through the B2 port and the reactant solution through the B3 port, and then carry out vortexing under the appropriate temperature. (B2:Bottole2, B3:Bottle3 Reference:Page4 Equipment configuration)
- Amount of liposome to prepare: 2 to 20 mL per run by automated single-run preparation and by automated continuous-run preparation up to 10 consecutive runs

Features of Automated Multi-functional Liposome Manufacturing Equipment

Varied automated preparation programs

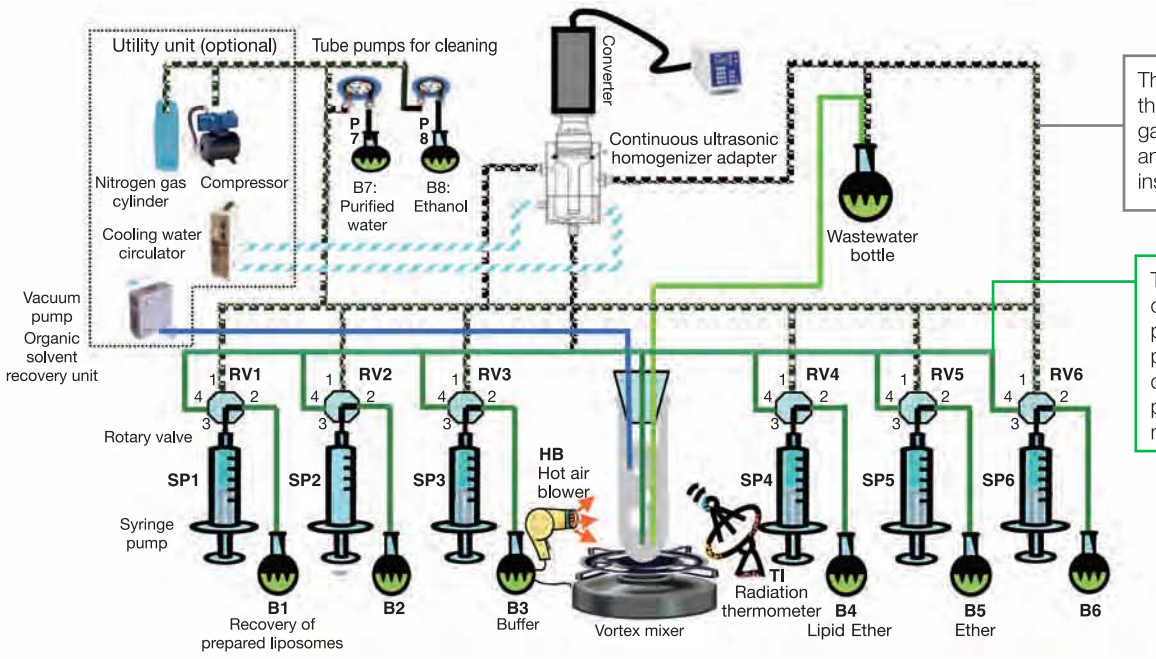
- Automated liposome preparation programs: Registration of 10 sets of preparation conditions each for up to 12 kinds is available.
- Bioreactor programs: Up to 15 user-created programs can be registered.
- Automated cleaning and drying programs: Details of operation, such as the cleaning liquid volume, drying duration, and number of runs, can be specified.

Equipment functions and features



- Ultrasonic converter
- Tube pumps for automated cleaning water and ethanol. Automated cleaning and drying programs are incorporated.
- Automated continuous/batch processing using water-cooled continuous ultrasonic homogenizer adapter
- Vortex mixer test tube (100/50 mL) equipped with hot-air heating and vacuum evaporation functions
- Large-amplitude vortex mixer vibrator plate capable of revolutions of 0 to 2,500 rpm and amplitude of 9 mm
- Two lines of sample bottles, 40-mL conical bottom bottle and 250-mL bottle, are available depending on the required volume.
- Maximum flow-rate adjustment knob for air and nitrogen gas
- Pressure adjustment knob for air and nitrogen gas
- A color touch panel enables simple operation of program creation and selection.
- Power switch
- Programs can be saved into a USB memory.
- Pinch valves, which are readily maintained and cleaned, are adopted.
- The rotary valve is switched to the direction of nitrogen gas, which pushes out the solution, thereby minimizing residual liquid.
- Supporting various applications and high-precision liquid delivery are available with syringe driver selectable between 1, 5, and 10 mL syringes, three aqueous solution lines and three organic-solvent solution lines

Equipment configuration



The common line for thedelivery of nitrogen gas, air, cleaning water, and ethanol is installed inside the housing.

Tubing for the delivery of user solutions pumped by syringe pumps, are installed outside the housing, providing good maintainability.

Aqueous solution (buffer, solution of soluble substances to be encapsulated)

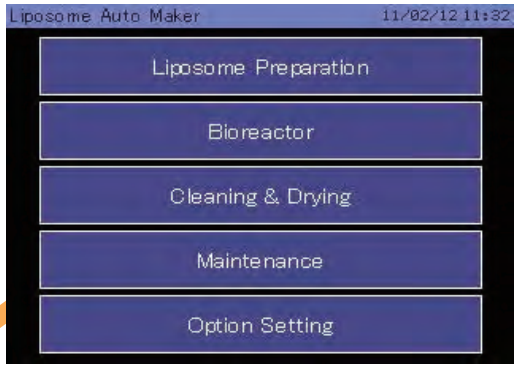
Organic-solvent solution (lipid ether and ethanol)

The Touch Panel Allows Simple Operation and Easy Creation of Programs

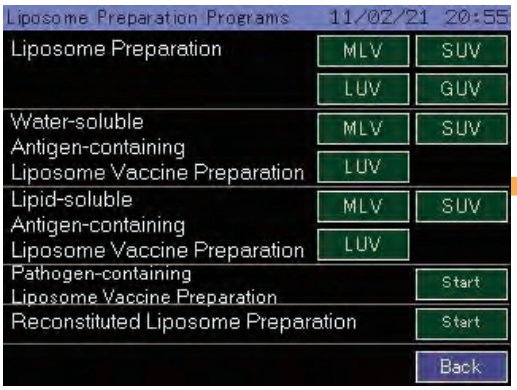
Touch panel operations for MLV preparation

(1) Touch panel screen when power is turned on

(2) Liposome preparation is selected on the Process selection screen



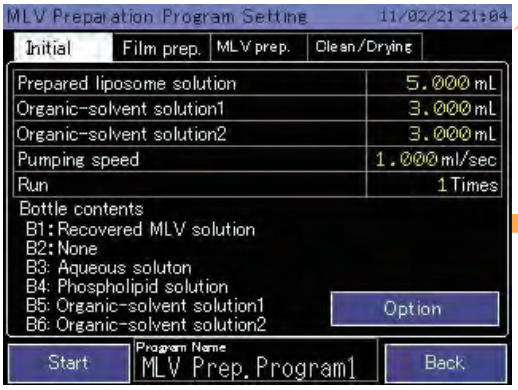
(3) MLV is selected for the liposome to be prepared



(4) A desired program No. for MLV preparation is selected

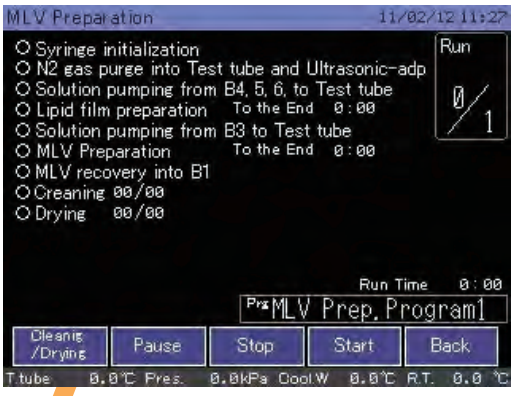
Up to 10 sets of preparation conditions can be registered

(5) Enter the amount to be prepared, number of runs, vortex conditions, etc.



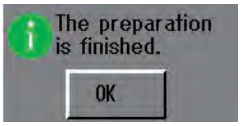
In a similar manner, enter the conditions of film preparation, MLV preparation vortex, cleaning, and drying, and then press the start button.

(6) The screen during MLV preparation



The MLV preparation program is started, displaying the screen during MLV preparation shown above. The running step is indicated by the lighting of a small circle at the beginning of the corresponding line.

(7) MLV is completed, and recovered in the bottle 1 (B1).



Other items to be set
Film preparation:
Number of vortex revolutions, duration of the vacuum level 1, 2, 3, and 4, and temperature
MLV preparation:
Regular and reverse rotation durations, regular and reverse rotation interval durations, temperature, and total duration
Cleaning and drying:
Selection among cleaning and drying intensity levels L, M, and H

Functions of Programmable Bioreactor

Features

- Automated operation combining vortex mixer processing with evaporation functions and continuous ultrasonic homogenizer
- Automated execution of the process of mixing, dispersion, reaction acceleration, concentration, and dispensing of six different solutions under nitrogen atmosphere, which are defined in user-created programs
- Non-manual operation is supported for the program instructions by selecting icons on the screen, and 15 programs can be registered.
- Automated cleaning and drying using either purified water or ethanol
- A part of the program or the whole program can be repeated for a specified number of times.

Features of component units

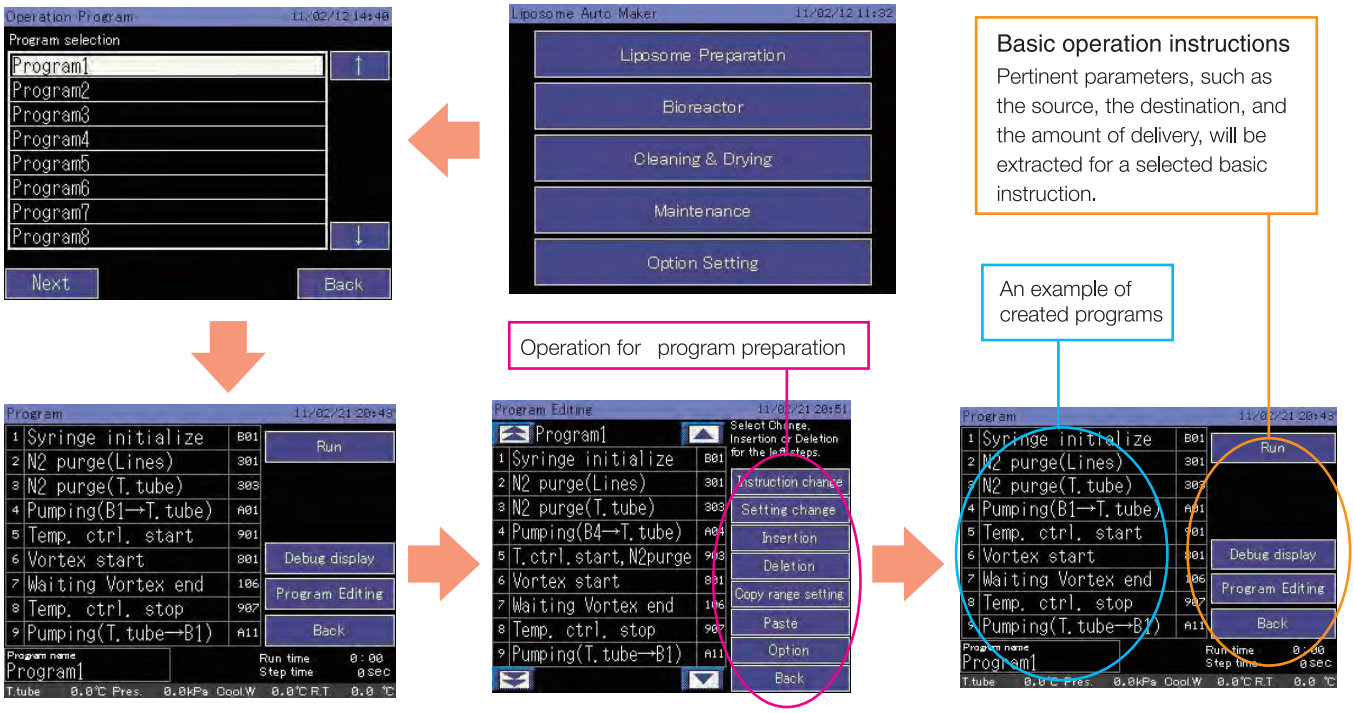
- Ultrasonic homogenizer

  A water-cooled continuous homogenizer adapter is incorporated, and six syringe pump units are used to deliver liquid for a wide range of applications, such as diffusion, emulsification, reaction acceleration, dispersion, and high-quality mixing and defoaming. Combination of continuous, batch, or circulation processing and vortex mixer processing is also available. Injection during vibration is also allowed.
- Syringe pump

  Stepping motors are used to control the syringe stroke length through 60 mm at the resolution of 24,000. Syringes are selectable from 1, 5, and 10 mL. Concurrent operation of syringe pumps at respective speeds is allowed, enabling synchronization of mixing timing and precision delivery of liquids. The syringe pumps can be used also as dispensers. Residual solutions remaining in the syringes are purged by nitrogen gas after syringe push-out.
- Vortex mixer

  Powerful mixing by 0 to 2,500 rpm of vortex revolutions and vibration of 9-mm amplitude are allowed, and test tube surface temperature is controlled by means of hot-air blowing and radiation thermometer. The vortex mixer can serve as a highly efficient evaporator by extending the surface area and evacuating the test tube. It can also serve as a continuous concentrator by combining syringe pumps.

Operation instructions



Specifications of Automated Multi-functional Liposome Manufacturing Equipment

• Automated liposome preparation program (optional)

Kinds of liposome: SUV, MLV, LUV, GUV, Functional liposomes, Reconstituted liposomes,
Liposome vaccines incorporating pathogens, Liposome vaccines encapsulating soluble antigens (MLV, SUV, and LUV), and
Liposome vaccines incorporating lipid-soluble antigens (MLV, SUV, and LUV)
Amount of preparation: Selectable among 2 to 20 mL per run
Number of continuous runs: 1 to 10
Preparation time: About 2 hours per run (including cleaning and drying process)

• Bioreactor

Six syringe pump units are used to control the liquid delivery to the vortex mixer having the evaporator function and to the continuous ultrasonic homogenizer unit. It is a fully automated bioreactor that enables users to create as desired a series of preparation programs by selecting operation instructions for processing and liquid delivery available on the touch panel.

• Operation

A touch panel is used for program selection and creation.

• Configuration

Syringe pumps : Six units
Syringes : 1, 5, and 10 mL

Vortex mixer : 0 to 2,500 rpm (regular and reverse rotation) with evaporator and hot-air heating
Ultrasonic homogenizer unit : Output 200 W,
and water-cooled continuous ultrasonic homogenizer adapter

• Gas displacement

Automated operation completely free of air by means of nitrogen displacement of the whole system is supported.

• Cleaning

Automated cleaning and drying using either purified water or ethanol (details of operation, such as the cleaning liquid volume, drying duration, and number of runs, can be specified)

• Required utilities

Dry compressed air: 0.2 to 0.5 MPa, 20 L/min
Nitrogen gas: 0.2 to 0.5 MPa or more. 25 L per run
Vacuum pump: 20 L/min, ultimate pressure 1kPa

Cooling water circulator
Organic solvent recovery unit

• Dimensions

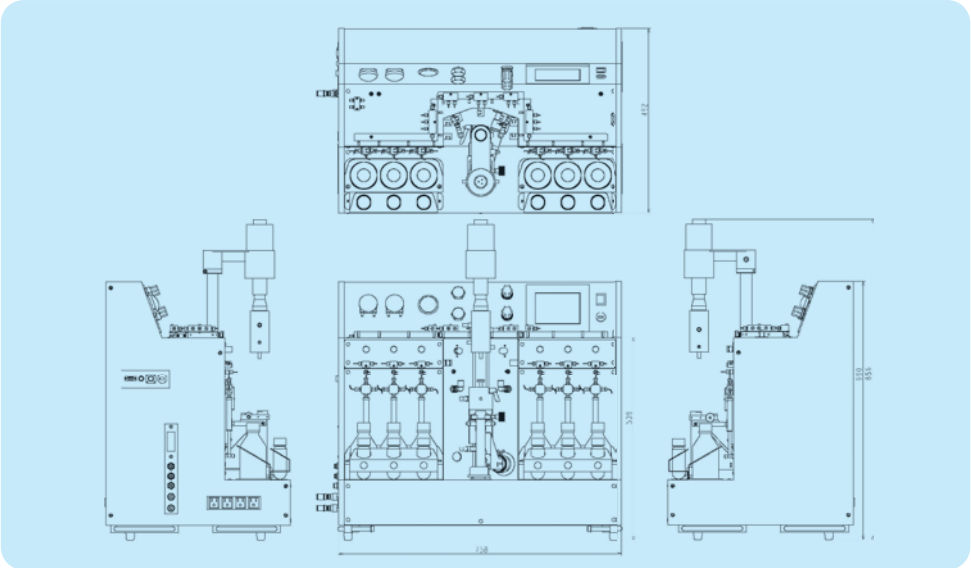
About 758 mm (W), 492 mm (D), and 690 mm (H) (excluding projections)

• Mass

About 95 kg (excluding the ultrasonic homogenizer power supply)

• Power source

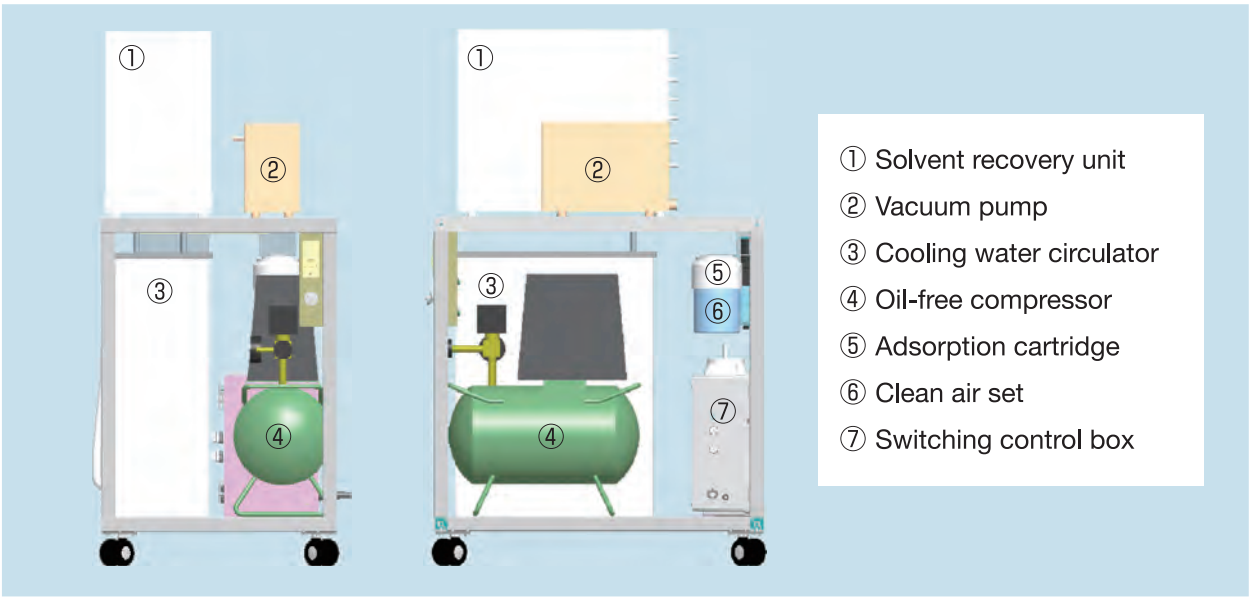
100 VAC, 50/60 Hz, 15 A(115VAC, 220VAC)



■Specifications of Utility Unit (Optional)

- Air compressor : Oil-less compressor, discharge pressure of 0.6 to 0.8 MPa, flow rate of 20 L/min
- Clean air system : Hollow fiber membrane air dryer plus filter regulator
  Atmospheric dew point of -10ºC, nominal filtration rating of 0.01 μm,
  purge volumeof 3 L/min
- Vacuum pump : Teflon diaphragm vacuum pump, pumping speed of 20 L/min,
  ultimate pressure of 1kPa
- Organic-solvent recovery unit :
  Water-cooled solvent recovery
- Cooling water circulator :
  Temperature control range of -20 to 20ºC (up to normal temperature)
  Circulation pumping speed of 12 L/min (max.)
- Control box : Continuous ultrasonic homogenizer adapter cooling-water control
  Hollow fiber membrane air dryer control
  Organic-solvent pathway control
- Dimensions : About 510 mm (W), 710 mm (D), and 1150 mm (H)
- Mass : About 50 kg
- Power source : 100 VAC, 50/60Hz, 10A (115VAC, 220VAC)

Note: A separate nitrogen gas cylinder is required for the nitrogen gas.



Partnership Project Approved by the Ministry of Economy, Trade and Industry as a "New Coordination Support Project" on June 26, 2009

Project name: Manufacturing and marketing business of multi-functional equipment for automated manufacturing of liposomes, such as those used for research and development

<Core enterprise>

Hashimoto Electronic Industry Co.,Ltd.

Research and development, manufacturing, and maintenance of the equipment

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<Partner enterprise: Reference>

Liposome Engineering Laboratory,Inc.

Research and development of application technologies of various types of liposome
Manufacturing method studies, market development, and quality evaluation

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