SepaBean™ machine T
Quick Operation Guide

This quick guide is intended to help new users of the SepaBean™ machine T get started quickly while familiarizing the users with the operation of the instrument's hardware and software, especially for setting separation method and elution gradient. There are two separation modes in the SepaBean App: normal phase and reverse phase.

In this guide, we will use normal phase separation as the example.

Operation Procedure

Step 1
System Power Up

Please make sure the instrument is conected to a wireless router and working well.

❶ Power on the instrument (the power switch is on the back panel).

❷ Login to the SepaBean App.

Step 2
Sample Preparation

Solid loading

Dissolve the sample in a proper solvent and then mix them wih silica gel or other inorganic particles. Evaporates the solvent with a rotary evaporator and then load the solid sample in a solid loading cartridge.

Liquid loading

Dissolve or dilute the sample with a solvent which has a weaker eluotropic strength. Meanwhile, dilute the sample into a volume as small as possible, no larger than 10% of the column volume of the flash cartridge to be used. For example, if the mobile phase to be used is hexane/ethyl acetate, choose hexane to dissolve the sample. In case the sample cannot be completely dissolved in pure hexane, then ethyl acetate could be increased in ratio.

Step 3
Method Setting

1 **Choose to start with normal phase separation or reversed phase separation**

On the main screen, touch the related button to go to the next page.

Nomal-phase separation

2 **Input the sample information**

Input one part of the sample information, including project number, CAS number of the sample, sample name, or chemical structure of the sample. Then touch the search button to find any available separation method in the built-in separation method database.

3 Input the TLC information for the sample

Choose the solvent used for TLC development of the sample.

SolventA:	Petroleum ether ▼
SolventB:	Ethyl acetate ▼

Note *Define the solvent which has stronger eluotropic strength as Solvent B. For example: if petroleum ether/ethyl acetate is used as the TLC development solvents, define petroleum ether as Solvent A, ethyl acetate as Solvent B.*

4 Input the ratio of the solvents

Input the percentage of the solvents used for TLC development.

91%	Solvent A is weaker in polarity
9%	Solvent B is stronger in polarity

## 5 Input the TLC results of the sample

According to the TLC results, input the retention factor (Rf) value of the target product and the adjacent impurity spots. Click a √ means this spot represents the target product.

Highly Recommend

Input the detailed TLC information so that SepaBean™ App will recommend a proper elution gradient for the following flash chromatography.

	RF1:	0.5	✓
	RF2:	0.35	✓
	RF3:	0.2	✓

The complete TLC information will help to generate gradient elution method, or you can skip it and set the gradient manually.
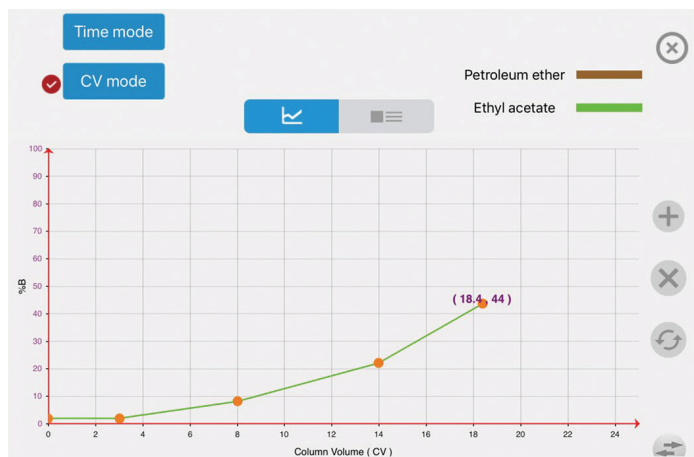
6 Separation parameter settings

❶ Input the sample loading amount (the unit could be switched between mg or g).

❷ The flash cartridge will be automatically recommended according to the sample loading amount, allowing the to user select a desired flash cartridge manually. The flow rate will be recommended automatically according to the related cartridge size, or the user could change it manually.

Sample loading	1	g	Total sample weight	1	g	Unit	◯ g
Flash column	iS-8101-0025 ▼		Flow rate	30	ml/min	Auxiliary column	◯ OFF

❸ Choose the detection wavelength. The default two wavelengths are 254 nm and 280 nm. Users can set the desired wavelength manually.

Note *The fraction collector will be triggered by the signal from wavelength channel 1 when the collecting mode is set to threshold collection.*

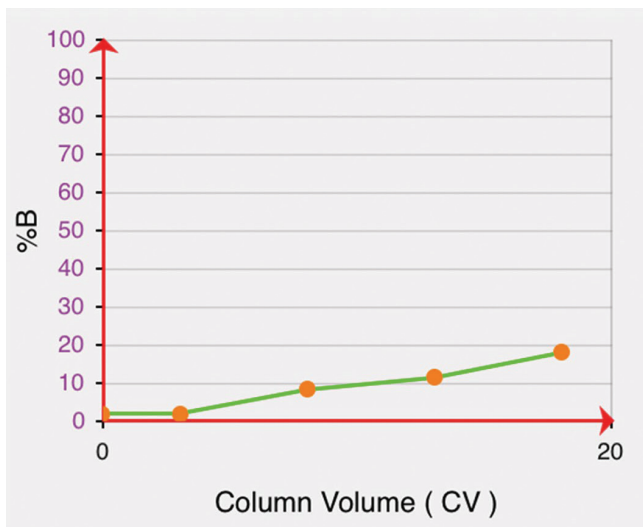❹ Choose a collecting mode: threshold collection is recommend and the value of the threshold could set as 10 mAu.

UV1 (Collection)	254	nm	UV2 (Monitoring)	254	nm
Collection mode	Threshold ▼		Threshold	10	mAu

❺ Insert the racks with tubes and set the collecting volume for each tube. It's recommended that the collecting volume be set 2 mL smaller than the tube volume.

6 Set the starting tube rack and starting tube number fraction collection. The default starting tube is the first one.

Volume	10	ml
Tube racks	Auto	▼
Starting tube tack	Tube racks 1	▼
Starting tube	1	

7 Edit the elution gradient: if the TLC information was inputted in details then a recommended gradient will be automatically generated. Otherwise users should edit the elution gradient manually. The recommended gradient could also be manually modified by the user.

Step 4
Tubing Cleaning

Clean all the tubing with Solvent B then Solvent A as the default setting. If the tubing cleaning was done before, then this step can be skipped.

Purge	⊗
1st. solvent:	Ethyl acetate
1st. washing time:	1 min
2rd. solvent:	Petroleum ether
2rd. washing time:	1 min

Washing

Step 5
Flash Cartridge Installation and Equilibration

Install the flash catridge and equilibrate the cartridge with default settings.
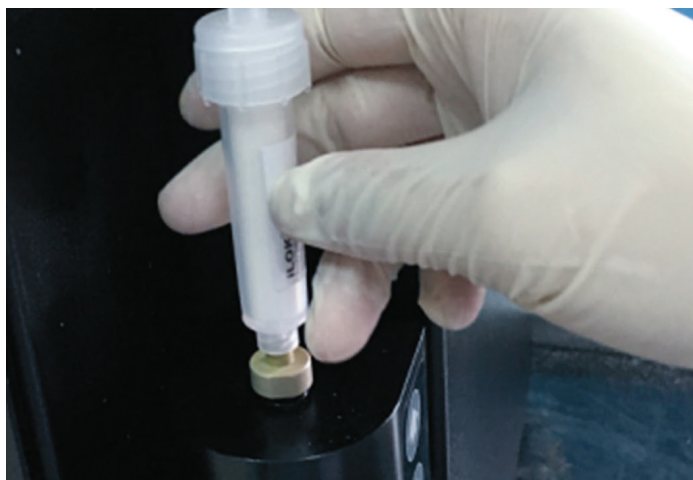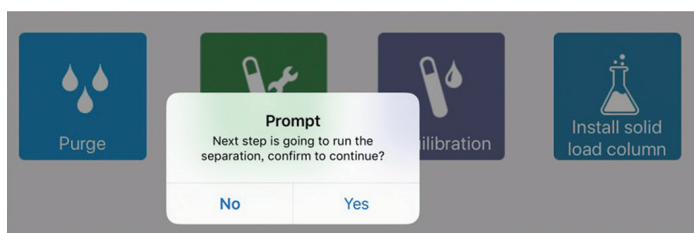

Step 6
Sample Loading

For solid sample loading, install the sample loading cartridge when equilibration is completed. For liquid sample loading, inject the sample on the top inlet of the smart column holder with an injector.

Step 7
Run Start

When sample loading is complete, the next step will start the separation.

Step 8
Post-run operations

When a run is finished, the user can choose to blow dry the flash cartridge as prompted by the SepaBean App. The duration for air pumping can be edited by the user. Then the user can choose to clean the system tubing with proper solvents.

Air purge		Purge	⊗
Purge Time:	1 min	1st. solvent:	Ethyl acetate
	11%	1st. washing time:	1 min
		2rd. solvent:	Hexane
		2rd. washing time:	1 min
End		Washing	Skip

Step 9
Run Report

A run report will be automatically generated when a run is finished. The user can check the run reports in the history record page.

History record — Methods

Project ID	Time	Operator		
				2019-07-15
Test	14:05:56	13685206385	Not shared	★★★★★
Test	13:42:25	13685206385	Not shared	★★★★★
Test	13:30:27	13685206385	Not shared	★★★★★

Run Report — Methods

Sample Info

Project ID:TEST   CAS:93-58-3
Quantity:10.0 mg

TLC Info

Solvent	Name	Percentage(%)
A	Hexane	91
B	Ethyl acetate	9

RF1 : 0.50	RF2 : 0.30

Settings

Flash column:iS-8101-0012   Flow rate:30.0
UV1:254 nm   UV2:280 nm
Collection mode:Threshold

Chromatogram

Gradient

NO	CV	Hexane	Ethyl acetate
1	0.0	100.0	0.0
2	10.0	70.0	30.0
Solvent statistics		167.17 ml	28.81 ml