# Instruction manual

Blotting System with Built-in Power Supply

WSE-4125 PoweredBLOT 2M

Version 1 November 29, 2018



# **Table of contents**

Int	roducti	on	1
Ins	structio	n manual	1
Op	eration	n precautions	1
Sa	fety sy	mbols	1
Pr	ecautio	ons when using	2
Ot	her pre	ecautions	3
1	Overv	riew	4
	1.1	Purpose of use	4
2	Confir	rmation When Unpacking	4
	2.1	Confirmation when unpacking	4
	2.2	Equipment configuration	4
3	Name	and function of each parts	5
	3.1	Main Body	5
	3.2	Accessories	6
4	Prepa	arations	7
	4.1	Usage environment	
	4.2	Preparation of experimental equipment	7
	4.3	Preparation of consumables	
	4.4	•	
5	Opera	ation	
	5.1	Preparation of reagents and experimental consumables	
	5.2	Pretreatment	11
	5.3	Setting of filter paper-membrane-gel	12
	5.4	Connection of the power supply	16
	5.5	Power supply	17
	5.6	After turning off the power	18
	5.7	Detection	19
	5.8	Cleaning and storage of the apparatus	21
6	Troub	leshooting	22
7	Mainte	enance	23
	7.1	Cleaning	23
	7.2	Inspection	
	7.3	Warranty	
	7.4	Consumables	
8	Specif	fications	26
9	Conta	act	26

#### Introduction

Thank you for purchasing WSE-4125 Powered BLOT 2M, an ATTO scienctific instrument. The product is delivered to you along with the device manual to support full utilization of the product in your laboratory. Not only first-time users of the product but also experienced users are asked to read and understand thoroughly this manual before use.

This manual is configured in a read-through style. Before using the device for the first time, please read this manual from beginning to end. Furthermore, this manual contains instructions and information on maintenance, the warranty, and service, in addition to the operation. Keep this manual at hand for effective use. Feel free to contact us for enquiries about your purchased product or the manual.

#### Instruction manual

Please read this manual thoroughly to become acquainted with the proper use of the product. After reading, keep this manual readily available for reference. If the product is to be relocated, make sure to include this manual with the product. If missing pages or binding errors are found, lost or damaged, a new copy will be provided. Please contact your sales agency or us and give the product name and model name. Every effort has been made to prepare this manual accurately and completely, but if any points of query, errors, or omissions are found, please notify us.

### Safety precautions

Correct operation is prerequisite in order to use this unit safely. Please read through this instruction manual before use, and do not use the unit until you fully understand the contents. How to use and the safety precautions listed on this manual are specific to usage of this particular unit only. Never use the unit for other purposes than those listed on this Manual. Any other usages of this unit than those listed on this manual would be strictly at your own responsibility.

The first-time user is asked to receive guidance from a person with correct knowledge and understand principle and method before use. Both the fresh users and experienced users keep this manual at hand for effective use. The only way to avoid electric shock and malfunction of the unit is to operate it correctly in accordance with this manual. Feel free to contact us for any questions about principle of electrophoresis, operation, maintenance and inspection.

### Safety symbols

The following symbols are used in this manual and on the product to assure and maintain safety in the use of the device.

Symbpl	Description
<b>Danger</b>	Failure to observe this precaution could lead to an imminent risk of death or serious injury.
Warning	Failure to observe this precaution could lead to risk of death or injury.
Caution	Failure to observe this precaution could lead to the damage of property.
	This symbol indicates important information.
	This symbol indicates tips and hint.
	This symbol indicates prohibited acts.

### Operation precautions

These precautions are necessary to prevent fire, electric shock, or other accidents and failures. It is essential to understand and observe these precautions. If the device is used in a manner other than that described in this manual, the built-in protective functions may fail to operate.



#### **Danger**

Power Connection	Do not use this type of power cable, of which terminals and plugs are deformed or corroded, or of which insulation cover is separated or damaged. It causes electrical shock or fire due to poor contact. Refrain from using the device and contact us. After turning off the device, disconnect the power cable from the receptacle. On unplugging the power cable, hold the plug, but do not pull on the cable.
No wet hand	Do not operate the device with wet hands. Also do not touch the power plugs and terminals with wet hands. It causes electrical shock or failure of the device.
Main body	Do not insert a foreign material into the device. It causes electrical shock or failure of the device. Do not use the device in wet condition. These causes electrical shock or failure. Clean all moistures up from surface of the device sufficiently when you use it.
Maintenance	Please quit using the device immediately if anything abnormal happens during device usage or if any abnormality/failure is suspected. Also, do not use the device if you find





usage or if any abnormality/failure is suspected. Also, do not use the device if you find any failure at inspection. These can cause electrical shocks or damage to the device. During the operation with blotter, please check if there is any abnormal sound or leaking or visually suspected troubles. Contact us if you detect any abnormality, trouble or failure.

Reagent

In operation of electrophoresis or blotting, it may use substances with deleterious substance, hazardous materials, and carcinogenic for Preparation of buffer solution, staining or bleaching operation.



Never contact it with human body directly, it causes accident or failure to the human body to cause death or burns. In use of chemicals, carefully read the handling cautions or warning of chemicals and protect your body with gloves and masks.



### Warning

Installation Location	Please avoid installation on places such as a wobbling table, tilted location, or heavily vibrating place. Install the Unit on horizontal places with safe and hard surfaces, such as a lab bench. It causes electrical shock failure by leaking liquid or falling down. Do not put anything on the device, it causes electrical shock by falling down.
Body	This device is not an explosion-proof structure. Install this device away from any places with the possibility of exposure to fire or flammable gasses.
$\triangle$	When the device is moved from a low-temperature area (lowtemp. room, cold out-door, etc.,) to a high-temperature area (warm room, etc.,), there is possibility of [Condensation], i.e. moisture in the air becomes water drops. If condensation happens within the device, get it dry sufficiently. It causes electrical shock or failure.
Power Connection	Set the maximum voltage value to 150 V and the maximum current value to 3 A when energizing the device. Using more than that may cause damage to the device.
Migration	Never move the device or touch the operating panels of power supply during operation. It causes electrical shock by flowing out of liquid. Cords may get entangled, which might cause rollover. Make sure to turn off the power switch and remove the AC adapter when moving the device.



Maintenance

Please be sure to turn the Unit power switch OFF and pull out the power cable before performing maintenance and cleaning. These might cause electrical shock.

Please ask us for periodical maintenance, inspection and calibration so as to maintain good performance and safety of the device.

Disassemble

Prohibition



Never disassemble or modify the Unit. Adjustment and repair work of the product should be done by our service staff in charge. When adjustment or repair is required, please ask our company. Any accident caused by your disassembling or modification would be outside of our responsibility.

Seal Group



Never peel off the Warning Seal. The seals indicate dangerous areas. If any seal comes off, please contact us.



### Warning

Seals

Never peel off nameplate seals. These indicate important information for maintenance and control of the Unit.



#### **Other Precautions**

Application	WSE-4125 Powered BLOT 2M is a laboratory equipment, not for medical use. Never use this device for diagnostic or medical purposes.
Export	Export of specific work and cargo are controlled by Foreign Exchange Laws and Cabinet Order/Ministerial Orders of Foreign Trade Control Laws and those controls are applied to this Unit. Even if the Unit is not applicable to the Cabinet Order, it is required to submit documents accordingly and if it is applicable, then obtain export license from the Ministry of Economy, Trade and Industry, and then submit the license to the customs office.  When you export our product, please confirm with your supplier or our customer service department in advance.
Trademark/ Copyright	Copyright approval is required when you make copy/reprint a part or whole of the Instruction Manual. Specifications of the Unit and contents of the Instruction Manual may be changed without an advanced notice.

### 1 Overview

### 1.1 Purpose of use

WSE-4125 PoweredBLOT 2M is used to transfer samples in a gel onto a membrane. Polyacrylamide gels of compact size (60\*60), mini size(90\*80) and wide size gel (140\*80) can be applied.

## 2 Confirmation When Unpacking

# 2.1 Confirmation when unpacking

Upon arrival of the product, please confirm whether the main body and accessories are packed correctly or if any defects exist. In case there is any inadequacy, deficiency, etc., please inform your supplier or our company immediately. Please perform your confirmation as above at the time of unpacking within one week upon arrival of the Unit. Confirmation after more than one week may result in not receiving compensation for defects or missing articles.

### 2.2 Equipment configuration

This product consists of a main body and accessories.

#### Main unit

Main body (Semi-dry Blotting system with power supply, model: WSE-4125 Powered BLOT2M)

Model	WSE-4125	WSE-4125 · M
Code No.	2322496	2322497
Main body	Powered BLOT 2M	
Power supply	Dedicated blotting power supply	
Membrane		WSE-4051 ClearBlot P Plus membrane
wembrane	-	85×90mm, 20/pk
		CB-09A filter paper
Filter paper	-	85X90mm 400/pk

#### Material

Anode plate	Platinum-plated titanium
Cathode plate	Stainless steel
Base unit	Acrylic plastic Acrylic plastic
Cover unit Power supply case AC adapter	Aluminum ABS

#### **Accessories**

Model	WSE-4125	WSE-4125 ⋅ M
AC adapter		1
Power Lead cable		1
Roller		1
Instruction manual		1

### 3 Name and function of each parts

### 3.1 Name and function of main unit

This unit consists of (1) Cover (2) Base and (3) Power supply

The cover, the base and the power supply are combined.









### (1) Cover

The cathode plate is attached and used in combination with the base.

It's connected to the **cathode (-)** of the power supply.

#### ① Cathode plate

The cathode plate is chemical resistant stainless steel electrode plate. By connecting with the cathode terminal of the power supply, the plate becomes cathode (-).

#### 2 Latch Handle

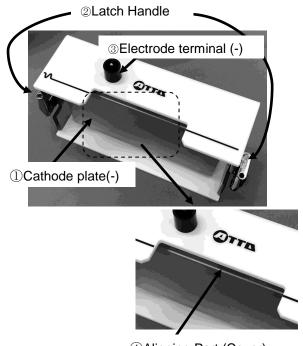
Holding the latch handle and the catch plate of the base, fix the cover and the base.

#### ③ Electrode terminal(-)

Connect with Power lead cable with safety terminal.

#### 4 Aligning Part (Cover)

Using for the adjustment of aligning of the cover and the base.



4 Aligning Part (Cover)

#### (2)Base

The base consists of the anode electrode plate and is used in combination with the cover.

It's connected to the anode (+) of the power supply.

#### ① Anode plate

The anode plate is a platinum-plated titanium electrode plate. By connecting to the power supply, the plate is charged anode (+).

#### ② Handle Catch Plate

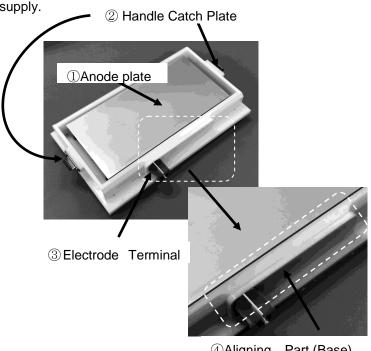
Holding the latch handle of the cover and the catch plate of the base, fix the cover and the

#### ③Electrode Terminal

Anode(+) terminal connected with Power Lead cable assembly(+).

#### 4 Aligning Part

Using for the adjustment of aligning of the cover and the base.



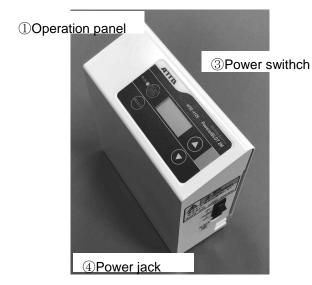
#### 4 Aligning Part (Base)

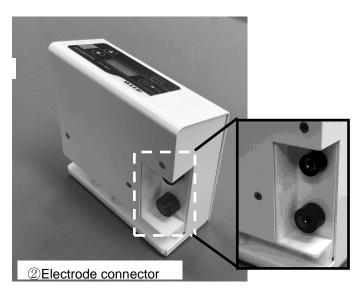
### (3) Power Supply

This is a power supply for energizing the main body of blotting apparatus under the condition of time and constant voltage set by the operation panel. By the timer function, energization stops automatically at the set time and alarm rings

If a short or open error occurs, turn off the power and make alarm ring.

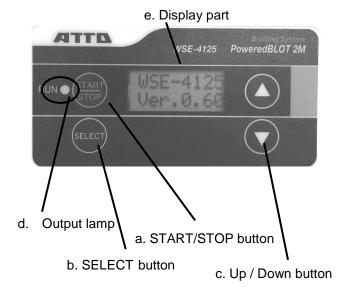
Two patterns of constant voltage output are possible: 12V, 2A and 24V, 1A.





#### 1 Operation panel

Displays the selection of output mode, set the energization time, start / stop energization operation, and display the selection mode, output status, and energization time.



#### a. START/STOP button

By pressing the button, it starts energization. The output lamp (RUN) flashes while the power is on. It stops when pressing stop during output.

#### b. SELECT button

Select the output mode. Press the button to change the output mode. The explanation of the output mode is described in "Types of output mode".

#### c. Up / Down button

Set the energization time, the unit is minutes. Press the Up / Down button to set the energizing time on the display. The range is 1 to 250 minutes.

#### d. Output lamp

Flashes green when the output energized. Flashes fast when a short / open error occurs. Other than that, the flashes are off.

#### e. Display part

The remaining time is displayed in the countdown method during the selected output mode, the set energization time, and during energization. The output mode is displayed on the top and the time is displayed on the bottom. The unit of time is minutes. The Display shows "End" when the power is turned off and "Short Err" or "Open Err" when a short / open error occurs.

#### f. Memory function

The energization time set for each mode is memorized. The last used mode and time are memorized, and displayed when the power is turned on.

#### g. Output limit function

The current value during electrophoresis with constant voltage output fluctuates according to changes in the electrical resistance value generated in the energized part (gel, buffer solution, etc.). When the maximum current value is reached during energization, it shifts from constant voltage output to constant current output.

\* There is no current value display function.

#### Output mode type

There are two output modes as shown in the table below.

The mode can be changed by pressing the SELECT button.

When using a blotting buffer that supports high-speed blotting and performing high-speed blotting, select "24V".

When using a normal blotting buffer, select "12V".

Mode/Output	Setting range	Set time
12V	1-250min.	15-60min.
24V		5-30min.

#### 2 Electrode connector

Connect to the upper / lower electrode terminals of the blotting main body. When the upper cover is fixed to the base and the upper / lower electrode terminals and the electrode connector part of the power supply part are pushed in, the power supply part is connected to the cathode and anode plates, allows energization.

#### 3 Power switch

When the AC adapter is connected, press the ON side to turn on the power. Press the OFF side to turn off the power. When set to ON, the output mode and set time are displayed on the display of the operation panel.

#### ④ Power jack

Connect the included AC adapter (24V DC 1.5A).

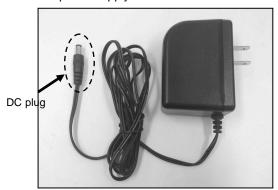


Be sure to use the AC adapter supplied with this system. If you use an AC adapter other than the one included, operation / output failure or damage of the power supply, deterioration or damage of the AC adapter, or fire may occur.

#### 3.2 Accessories

#### AC adapter (24V DC 1.5A)

AC adapter (24V DC 1.5A) AC adapter that can be used from AC100V to 240V. Supply 24 V DC to the power supply.



The AC adapter of AE-6685 and WSE-4110 cannot be used.

#### Roller

Used to remove air bubbles remaining , and excessive blotting buffer between membranes, filter papers and gels.



#### **Extension cord**

If it is difficult to plug the AC adapter into an outlet, use it by plugging in the AC adapter power plug.



# 4 Preparations

### 4.1 Usage environment

This chapter describes the preparation procedure before using this

Location	For indoor use only
Environment of usage	5-40°C • 5-70%RH (no condensation)



Never install the unit in the atmosphere of flammable gases. This can cause explosion or fire since the unit is not of an explosion-proof structure. Install the unit in the environment where no flammable gas is contactable. Never install the unit in a corrosive gas atmosphere. This can cause conductor corrosion or bad connector contact in the product. This, in turn, may cause malfunction, trouble or fire. Never install the unit in an environment where a lot of dust and dirt exist. Dust and dirt will stick to the unit and can cause electrical shock, fire or other problems.



Never use the unit in areas where strong magnetic or electric field exists or where a lot of waveform distortion or noise exists. These might cause malfunction. Never install the unit where a direct sunlight hit, temperature suddenly varies or humidity is high. Never use it if condensation happens. Please use the unit inside the room. This product has been designed to ensure safety and performance under the environment described in the Environment for Use.

### 4.2 Preparation of experimental equipment

#### **Shaking apparatus**

Used for dyeing and reaction of gels and blotted membranes. ATTO WSC-2400 See-saw Shaker ATTO

#### Gel documentation system

Used for color and luminescence image of gel and membrane after blotting.

ATTO Printgraph series (fluorescence)

ATTO LuminoGraph series (luminescence)

### 4.3 Prepare the following consumables

Prepare membrane and filter paper matched with gel size.

	Membrane	Filter paper	For Membrane Seal
For Compact gel (60x60x0.75mm thickness)	WSE-4050 ClearBlot- P+membrane (65×65mm)	CB-06A Filter paper (65×65mm)	Pitatt Clear
For mini gel (85x90x1mm tmckness)	WSE-4051 ClearBlot P+membrane (85×90mm)	CB-09A Filter paper (85×90mm)	Pitati Cieai
For wide gel (85x150x1mmthickness)	WSE-4053 *1 ClearBlot P+membrane (260mm×3m)	CB-20A *1 Filter paper (200×200mm)	

<sup>1.</sup> In the case of wide gel (140 x 80 mm), cut the membrane (WSE-4053) and filter paper (CB 20A) to  $150 \times 85$  mm.

\* The following table shows the number of filters should be used for the blotting buffer.

Blotting buffer	Filter paper, the number of filter paper
AF-1460 FzBlot	Cathode X 3,
AE-1400 EZBIOL	Anelectrode X 3
AE-1465 EzFastBlot	Cathode X 2-3,
AE-1405 EZFASIBIOI	Anelectrode X 2-3
WSE-7210 EzFastBlot HMW	Cathode X 3,
VVSE-7210 EZFASIBIOL HIVIVV	Anelectrode X 3
WSE-4055 QBlot kit	None

<sup>\*</sup> The number of filter paper is for using our 0.9mm thickness product. When using filter paper of different thickness, please refer to the above number and adjust it to the same thickness.

### 4.4 Preparation and preparation of reagents

1. Necessary reagents

Prepare the following reagents according to your purpose.

- 1) Blotting buffer
- 2) Primary antibody and labeled secondary antibody such as enzyme and fluorochrome
- 3) Blocking reagent
- 4) Wash buffer
- 5) Detection reagent
- 6) Reprobe reagent

We sell the products described in "7.4. Consumables". Please use them depending on the application. Contact us for more product details.

### 2. Preparation of reagents

Please prepare depends on the instruction manual of each reagent to be used and the dissertation etc. The following is a brief description of reagent preparation when using our related products. For details, please refer to the instruction manual attached to each product.

	Model	Product name	Method for preparation	Preparation amount (1 mini gel)	
Pre-wet PVDF mem- brane	WSE-4055	QBlot kit	Dilute Gel Wash Buffer 1/5 with distilled water.	50mL	
	AE-1460	EzBlot	Add 25 mL of methanol to each of the unopened A to C bottles.	A-C around 50mL each	
Diatting	AE-1465	EzFastBlot	Dilute to 1/10 with distilled water.		
Blotting buffer	WSE-7210	EzFastBlot HMW	Dilute to 1/5 with distilled water.	Around 200mL	
	WSE-7055	EzRun TG	Dilute to 1/10 with distilled water. Add 5-20% of methanol depending on the purpose.	Around 200mL	
	AE-1475	EzBlock Chemi	Dilute to 1/5 with distilled water.	Prepare 650 uL of diluted solution per 1cm <sup>2</sup> of membrane. About 50 mL for mini	
Blocking buffer	AE-1476	EzBlock BSA	For AE-1476 / 77, add 1/100 vol-		
bullel	AE-1477	EzBlock CAS	ume of attached Tween 20.	gel	
Wash buffer	WSE-7230	EzTBS	Dilute to 1/10 with distilled water. Add surfactants such as Tween	Prepare about 300	
	WSE-7430	EzPBS(-)	20 and Triton X-100 to a final concentration of 0.02 to 0.5% when it's necessary.	mL of diluted solu- tion.	
Detection reagent (coloring)	AE-1490	EzWestBlue	Use the undiluted solution.	Prepare 100 uL per 1cm <sup>2</sup> of membrane. About 10 mL for mini gel	
Detection reagent	WSE-7110	EzWest- LumiOne		For minigels, prepare 5 mL per plate.	
(luminescen ce)	WSE-7120	EzWestLumi Plus	Mix Reagents A and B 1: 1 just before detection.	In case of mini gel, prepare 2.5 mL each of A and B per sheet.	
Reprobe reagent	WSE-7240	EzReprobe	Use the undiluted solution.  Add 0.6g to 100mL of the attached "Enhancer" if necessary.	Prepare 30 mL per membrane of mini gel size (85 x 90 mm). Also, prepare wash buffer.	

# 5 Operation

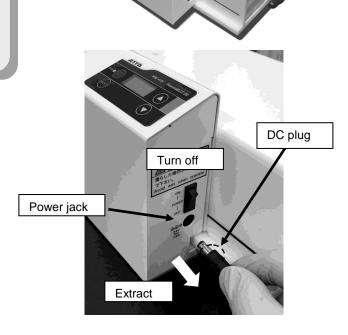
# 5.1 Preparation of equipment

As shown in the right figure, when the main body of the blotting device and the power supply are combined, remove each as follows.

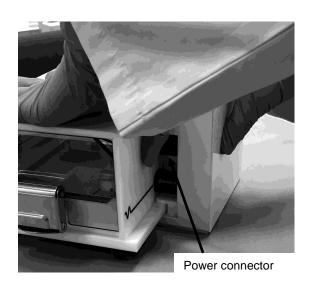


Remove the AC adapter from the outlet before removing the DC plug. There is a risk of injury such as electric shock.

Set the power switch of the power supply to OFF. Also, remove the AC adapter DC plug from the power supply.

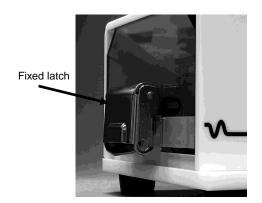


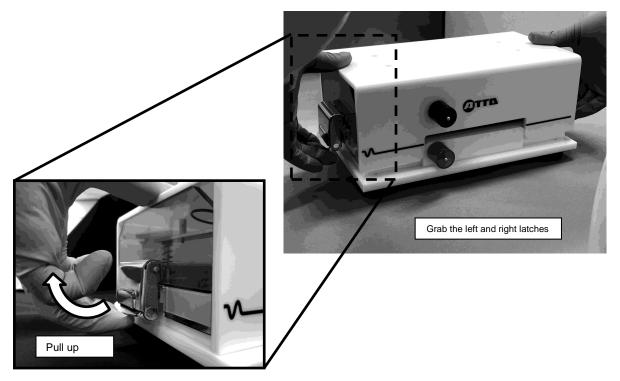
Remove the power supply from the blotting main unit. As shown in the photo on the lower right, hold the blotting device, put your finger on the top of the power supply, and pull it horizontally to remove.





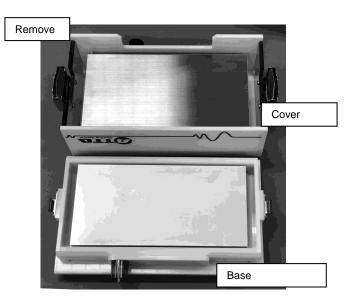
Grab the left and right fixed latches on the cover, and while lifting the lever, lift the cover upwards.





Remove the cover from base.

From this point, the cover needs to be removed until the settings of filter paper, membrane and gel is completed.



#### 5.2 Pretreatment

Prepare containers for membrane, filter paper and gel pretreatment respectively.

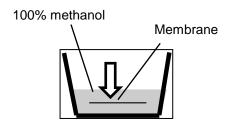
- 1.Pretreatment of the membrane
- 1 Pretreatment of the membrane (Hydrophilic)

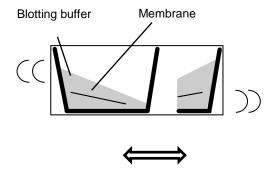
  Soak Clear Blot P Plus Membrane in

  100% methanol at the bottom and to
  saturate it for approximately 20seconds.

  Then immerse this membrane in buffer
  and saturate it for 5 to >10 minutes.
  - \* Never touch the membrane
    with the bare hands. Handle it only
    with hands covered in clean
    experimental gloves or pick it up
    with tweezers.
  - \* This pretreatment of the membrane should be completed before the completion of the gel electrophoresis.
  - \* Since the membrane tends to repel blotting buffer at first, make sure the membrane is saturated with buffer by shaking the container rather strongly.

    Insufficient saturation of the membrane may cause uneven blotting.
  - \* The membrane used should be the same size as the gel.







When using filter paper other than CB-06 / 09A / 20A filter paper, use one with a thickness of 0.9 mm / sheet. When using filter paper of other thickness, adjust the number of sheets to the same thickness referring to the number of filter sheets used on page 10. The size of the filter paper is same size as the gel. If the thickness or size is different, uneven blotting or "Err" may be displayed on the power supply, blotting may not be possible.

#### 2 Pretreatment of the filter paper

Prepare the required number of absorbent paper (filter paper). Soak all the filter papers in blotting buffer for at least 10 seconds.

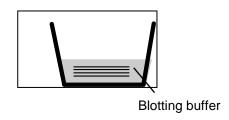
- \* This pretreatment of the absorbent (filter) paper should be completed before the completion of the gel electrophoresis.
- \* To completely soak the sheets of filter paper in buffer, use a sufficient volume of blotting buffer.
- \* The filter paper may melt if soaked for a long time.

#### 3 Pretreatment of the filter paper

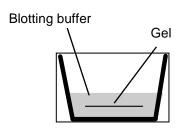
After electrophoresis, remove the gel from the glass plate. When taking out, cut off excess gel, such as the lower end of the gel and the sample well at the upper end of it. Then, rinse with blotting buffer to remove running buffer and gel fragments attached to the gel.

If it is left for a long time (more than 5 minutes), the gel may swell, and low-molecular-weight proteins may become detached from the gel, resulting in reduced blotting efficiency. Also, uneven blotting or "Err" may be displayed on the power supply, and blotting may not be possible.

#### 2.Pretreatment of the filter paper



#### 3.Pretreatment of the gel



### 5.3 Setting of filter paper-membrane-gel

Drop approximately 1 mL of blotting buffer on the anode electrode plate.



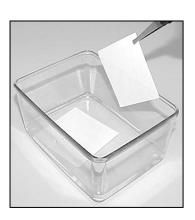
During the operations, make sure to wear clean experimental gloves on both hands.

Drop of Blotting buffer approximately 1 mL

Take out the filter papers from blotting buffer.



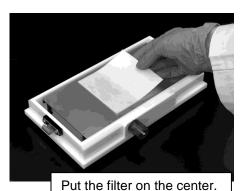
Do the same when taking out the filter paper after this.



For one gel blotting, place the filter paper in the center of the anode plate.



When blotting of one gel, be sure to place the filter paper in the center of the anode plate. If the filter paper is close to the edge of the plate, the blotting may be uneven or the power supply may display "Err" and the blotting may possibly be failed.





When blotting of two gels, be sure to place the filter paper evenly on the anode plate. If the filter paper is close to the edge of the plate, it may cause uneven blotting.

Stack the soaked membrane on the filter paper, fitting the four sides of the filter paper.

\*Be careful not to put air bubbles between the filter paper and the membrane.



When using two gels, put them evenly on the plate.

Hold the side of the membrane with your finger to avoid moving the layered sandwich. Push out any air bubbles and excessive blotting buffer by blotting roller.

Repeat rolling the roller in the opposite direction.

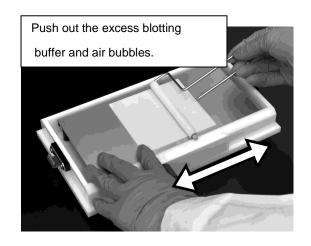


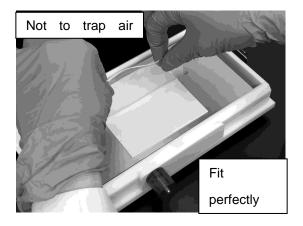
Please follow the above procedure to prevent from air bubbles remain, otherwise the remaining part cannot be blotted.

\* The blotting buffer oozes from the filter paper, but there is no problem. Drop about 1mL of blotting buffer on the membrane.

Layer the rinsed gel with blotting buffer on the membrane.

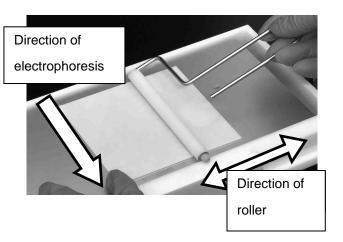
- \* Please put the whole gel so that it does not protrude from the membrane.
- $\*$  Be careful not to trap air bubbles between the membrane and the gel.
- \* Please do not put the gel on the membrane again. Just by touching the gel with the membrane, it may cause the sample to be blotted on the membrane.





Hold the side of the gel with a finger to avoid moving the filter paper and membrane. Push any air bubbles and excessive blotting buffer out by rolling a roller from one side of the gel where the finger is pressed toward the other side. Please move the roller at right angles to the direction of electrophoresis.

\* Press the roller lightly. If Moved with strong force, it may cause the gel to slip or break.



Layer the filter paper soaked in blotting buffer on the gel, fitting the 4 sides of the filter paper together without air bubbles.

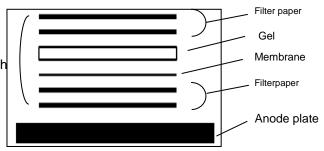
Sandwich

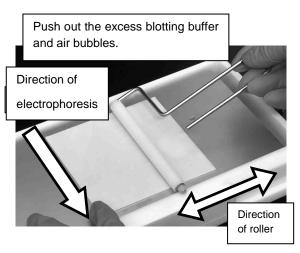
The filter, membrane and gel are now stacked as shown on the right picture. Hereafter, this structure is called as "sandwich".

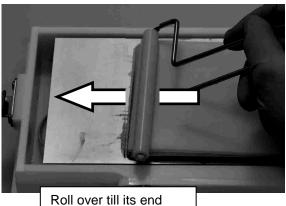
Hold the side of the membrane with a finger to avoid moving the layered sandwich. Push out any air bubbles and excessive blotting buffer by rolling a roller from one side of the membrane where the finger is pressed toward the other side.

Repeat rolling the roller in the opposite direction.

- \* Excessive blotting buffer will seep from the paper, but it is not a problem.
- \*Weak contact between the gel and the membrane may cause an obscure blotting pattern.
- \* Do not stop moving the roller at the end of the sandwich, roll over till its end.
- \* Do not stop moving the roller at the end of the sandwich, roll over till its end.

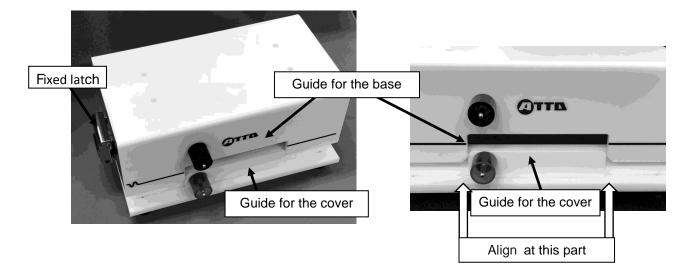






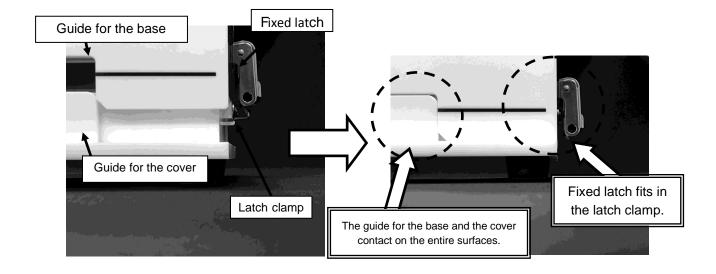


Hold the cover by grasping the left and right fixed latches of the removed cover, and pull up the levers of the fixed latch and align 'the guide for the cover' and 'the guide for the base'. Place the cover on the base.



When the cover of "guide for the base" and the base of "guide for the cover" are in close contact, release the fixed latch lever.

The fixed latch engages with the latch clamp and the cover is fixed.

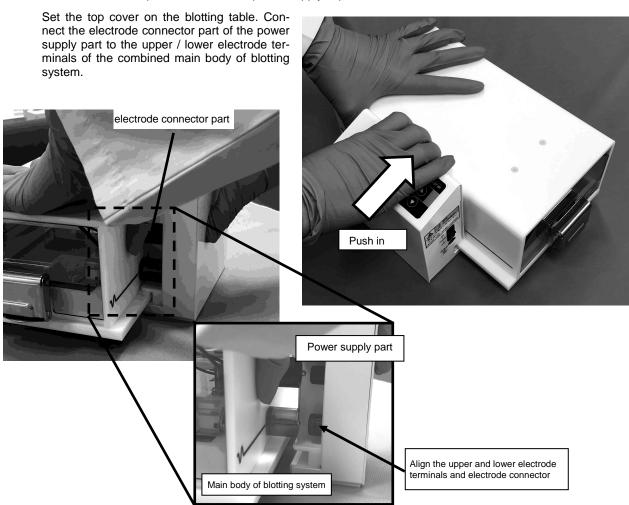




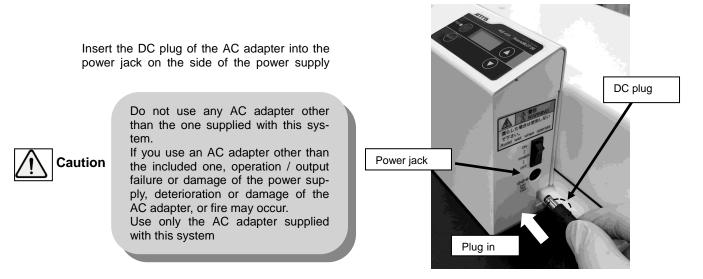
Do not remove the cover until complete the blotting. If you remove the cover, the filter paper may come off the cathode plate, and the gel and membrane may be dislocated. Once the gel and membrane come in contact, the sample appears on the membrane, causing the results to be disturbed.

### 5.4 Connection of the power supply

Make sure that the power switch of the power supply is pushed to the side which indicated as OFF.



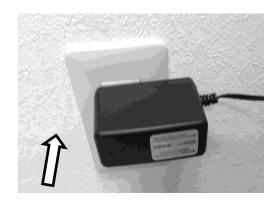
Visually align the position of the electrode connector on the power supply with the upper / lower electrode terminals of the blotting system, and then push in the power supply.





AC adapter of AE-6685 and WSE-4110 cannot be used.

Connect the power plug of the AC adapter or extension cord to a power outlet.



### 5.5 Power supply

Push the power switch of the power supply where "ON" is displayed to the side.

- \* At this time, the previous setting condition is displayed by the memory function.
- \* When blotting under the same conditions as the previous setting, it is not necessary to set again.

Select the output mode.

Press the select button to set between "12V" and "24V" output modes.

Each time the select button is pressed, it switches between "12V" and "24V".





When setting the blotting buffer, mode and energization time, please refer to the below table;

_				
		Filter paper	Output condition and	
Blotting buffer	Gel	Number of filter	energization time	
		paper	12V	24V
AE-1460 EzBlot	Handmade gel ATTO precast gel	Cathode, Anelectrode X 3 each	30-60min.	-
AF 1465 FyFootPlot	Handmade gel	Cathode, Anelectrode X 2 each	20-60min.	10-25min.
AE-1465 EzFastBlot	ATTO precast gel			5-15min.
WSE-7210 EzFastBlot HMW	Handmade gel	Cathode, Anelec- trode X 3 each	30-60min.	15-30min.
TVOL 7210 LZI dStBlot I IIVIVV	ATTO precast gel	trode x 3 each		
WSE-4055 QBlot kit	Handmade gel		15-30min.	5-10min.
	ATTO precast gel			

<sup>\*</sup>The number of filter paper is when using our 0.9mm thickness product. When using filter paper of other thickness, refer to the above number and adjust it to the same thickness.



The optimal transfer time should be determined by preliminary experiments. To optimize the blotting efficiency of the target sample, it is necessary to examine transfer buffer and transfer time. By increasing the blotting time, generally increases the blotting efficiency. If blotting is performed for a long time, heat may be generated and the filter paper or membrane get dried.

Set the time.

The output time can be changed by pressing the "Up / Down button" on the operation panel (see the table above).



Up/Down button

By pressing the "START / STOP" button, it starts energization. The output lamp flashes. The display during energization shows the remaining time.

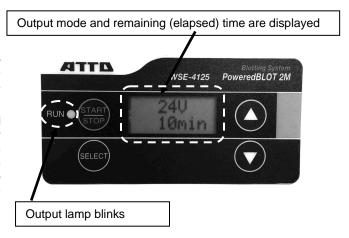
START/STOP button

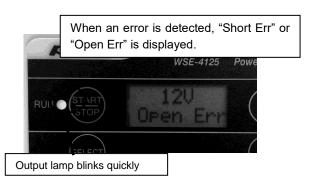


While the power is on, the output lamp blinks. The time and mode of output are displayed.

The remaining time is displayed through countdown. If the SELECT button is pressed while the power is on, the set time is displayed only while the button is pressed.

- \* When the START / STOP button is pressed while power is being supplied, the power supply is stopped. Press this button again to stop and restart during energization. In that case, the elapsed time till the pause gets reset, so please set the remaining time when restarting.
- \* If an abnormality (short or open error) is detected while the power is on, the power is stopped with an alarm, and "Short Err" or "Open Err" flashes on the display. The output lamp changes from flashing to flashing quickly. Turn off the main switch and unplug the AC adapter from the outlet. Refer to the next page and "Troubleshooting" to take appropriate action.





Open error (No load)	Both electrode connectors are not pushed in to the end.	Push in the electrode connector.
When current stops flowing "Open Err"	Both electrode connectors are not pushed in to the end.	Check the number and thickness of the filter paper. If filter paper other than our products is used, use one with a thickness of 0.9 mm. When using other thickness of filter paper, adjust the number of filter papers to the same thickness, please refer to the number of filter papers used on page 10.
	Disconnection	Stop using immediately and contact us (check the back cover)
Short error (short circuit)	The electrode plug is wet.	Wipe off any water adhering to the electrode plug.
When excessive cur-	Inside of the power supply is wet.	Stop using it immediately and contact us (check the back cover).
rent flows	There is a possibility of circuit failure in the power supply.	
"Short Err"		

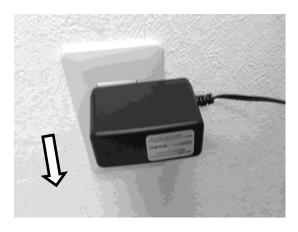
### 5.6 Completion of energization

When the set time elapses, power is automatically turned off, "End" is displayed, and alarms.

Press the power switch on the power supply to the side that is displayed as OFF. Check the display and output lamp are turned off.

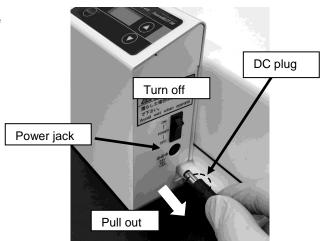


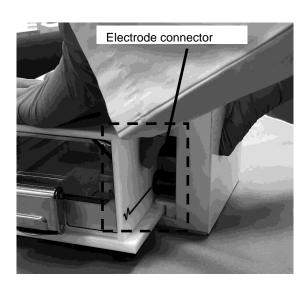
Remove the AC adapter or extension cord power plug from the outlet. Do not leave the power plug plugged in.

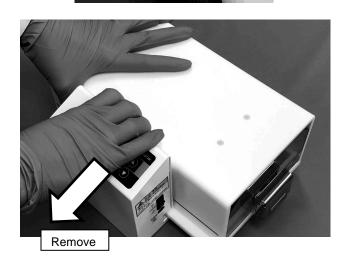


Pull out the DC plug of the AC adapter from the power jack on the side of the power supply.

Remove the power supply from the top cover / base. As shown in the photo on the lower right, hold the main body of blotting, put your finger on the top of the power supply, and pull it horizontally to remove.







Grab the fixed latches on the left and right of the top cover and lift the top cover upward while pulling up the lever.

\* The entire filter paper or sandwich may stick to the cathode plate. When lifting the top cover, be careful not to drop the sticking filter paper.





Please always wear clean laboratory gloves on both hands when handling the membrane.

Remove the filter paper and gel from the top of the sandwich and collect the membrane. Proceed to detect and stain target molecules by antigen-antibody reaction.

\* Filter paper cannot be reused. It may cause blotting unevenness and lower efficiency while blotting.



#### 5.7 Detection

The experimental flow of blocking, antibody hybridization, and detection is described below. For details, please refer to the instruction manual of the reagent and the reference papers.

#### 1. Blocking

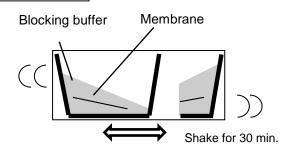
Immediately soak the blotted membrane in blocking buffer and shake at room temperature for 30 minutes (50 mL/gel).

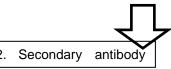
\* If blocking time is prolonged (1 hour or more), it will cause over blocking.

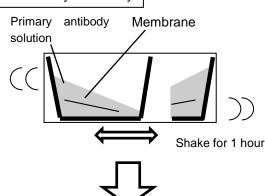
#### 2. Antibody reaction

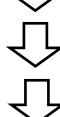
- Dilute the primary antibody in EzTBS (TBS-T) or EzPBS (PBS-T) containing 0.1% Tween (10 mL / gel).
- \* Dilution of antibody varies depending on antibody. Please refer to the instruction manual attached to each antibody. Generally, several hundred to several thousand times dilution.
- \* Blocking buffer may be used for antibody dilution.
- 2) Pour the antibody solution of 1) in a container one size larger than the membrane, and react with the sample while shaking for 1 hour at room temperature.
- \* Reaction time and temperature of antibody vary depending on antibody. Please refer to the instruction manual of each antibody. In general, the reaction is carried out at room temperature or 37 ° C for 30 minutes to 1 hour, or overnight at 4 ° C.
- 3) Discard the antibody solution, add 50 mL of TBS-T or PBS-T, and shake for 5 minutes (washing). Repeat the washing process three times or more.
- 4) Dilute the HRP-labeled secondary antibody with TBS-T or PBS-T (10 mL / gel).
- \* Dilution of antibody varies depending on antibody. Please refer to the instruction manual of each antibody. Generally, it is diluted by several thousand to several ten thousand times.
- \* If background is too high, increase antibody dilution rate or add blocking agent to antibody dilution at a concentration of 1/10 the normal concentration.





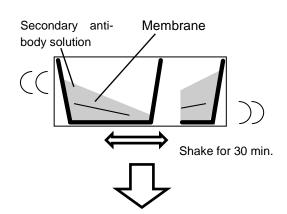






Washing for 5 minutes (3 times or more)



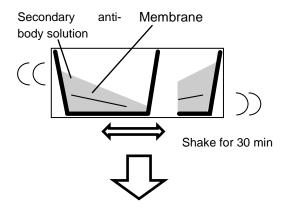


- 5) Add the antibody solution of 4) to the membrane after washing and allow to react while shaking for 30 minutes at room temperature.
- \* Reaction time and temperature of antibody vary depending on antibody. Please refer to the instruction manual attached to each antibody. In general, react for 30 minutes to 1 hour at room temperature or overnight at 4 ° C.
- 6) Discard the antibody solution, add 50 mL of TBS-T or PBS-T, and shake for 5 minutes (washing process). Repeat the washing process three or more times.
- \* Insufficient washing may result in high background.

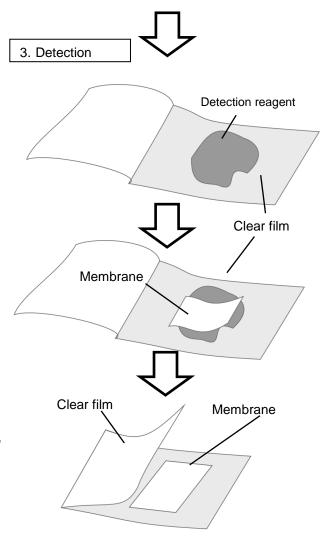
#### 3. Detection

This following describes chemiluminescence detection using WSE-7110 EzWestLumiOne as a HRP substrate reagent.

- 1) Pour the substrate reagent in a clean container one size larger than the membrane.
- \* Instead of the tray, you can also spread and use transparent films such as "ATTO pitatto clear" and wraps.
- Immerse the washed membrane in the substrate reagent for a few seconds. Make sure that the reagent is spread entirely the membrane.
- \* If the membrane does not immerse in the reagent uniformly, it may cause the foaming background or unevenness.
- 3) Seal the membrane with a transparent film or plastic wrap to prevent air from entering.
- \* If air gets in between the membrane and the film, it may cause foaming background and unevenness.
- \* When wrapped the membrane, if there are wrinkles on the surface, wrinkles background or unevenness may occur.
- Acquire the chemiluminescent image of the membrane using a chemiluminescent imaging system.



Wash for 5 minutes (3 times or more)



### 5.8 Cleaning and storage of the apparatus

#### 1. Cleaning of equipment

Wash the blotting device with running water before the blotting buffer dries. The upper and lower electrode plates are washed by using a soft sponge with a neutral detergent, and washing away bubbles completely, rinse with pure water. After washing, let it dry naturally.

The cathode electrode plate may remaind a white mark trace of filter paper, but please wash it gently with a soft sponge. If it is immediately after use, you can wash it by simply rubbing lightly.

Never wash the power supply and AC adapter with water. If it gets dirty, wipe it gently with a mild cloth diluted with water and a mild detergent. Do not use other organic solvents.

- \* Uneven blotting may occur if the anode and cathode electrode plate are heavily soiled.
- \* Do not push strongly the cathode electrode plate. The inner blade mechanism may be deformed.



Caution

Clean the upper cover and lower base after removing the power supply.

#### 2.Strage of the equipment

Do not store in direct sunlight, high temperatures or where there is a possibility of being exposed to corrosive gases.

### 6 Troubleshooting

In order to solve problems, it is necessary to confirm not only the device but also all operations from electrophoresis to detection operation such as electrophoresis gel, blotting buffer, detection reagent and so on. In addition to the items described in this chapter, also check the instruction manuals of the equipment and reagents used in each operation.

Symptom	Cours	Countermocoure		
- Cymptem	Cause	Countermeasure		
	Incorrect buffer	Confirm the composition of the blotting buffer, and if it is differ-		
	composition	ent or wrong, re-prepare fresh blotting buffer.		
	Incomplete connection of	Duck the newest comply into the ten cover / been until the class		
	an electrode connector	Push the power supply into the top cover / base until the elec-		
	to the power supply	trode connector stops.		
No power.		Make sure that filter paper and/or membrane are completely		
Voltage reading	Insufficient pretreatment	soaked in each blotting buffer during pretreatment.		
on power sup-	of the filter paper or	For protein blotting, soak the methanol-treated membrane in		
ply is zero	membrane.	blotting buffer but it will be float at first, so make sure that the		
when switched		shaking is enough strong to sink the membrane.		
on.		Immediately stop the use of this device, and contact us		
	Broken wire	(Please contact the customer service representative men-		
Voltage output		tioned on the back cover)		
is stopped while "error" is	Filter paper is reused.	Filter paper is not reusable. Always use new filter paper.		
displayed on		Check the number and thickness of the filter paper. When		
power supply.	Insufficient contact be-	using filter paper other than our product, use one with a thick-		
Politor outpers):	tween the cathode plate	ness of 0.9 mm. When using filter paper of other thickness,		
	and the sandwich.	,		
	The gal was socked in	the number of filter sheets used.		
	_	Do not soak the gel before blotting for a long time (more than		
	<u> </u>	5 minutes) in the blotting buffer.		
	long.	In the case of high molecular weight proteins, the transfer effi-		
	Blotting is not processed			
Missing bands				
	F F			
		by about 1.5 to 2 times.		
		After stacking the filter paper, gel, and membrane, remove		
David ()	Poor contact between the	excess blotting solution and air bubbles by rolling over with		
Band flows	membrane and the gel.	using the blotting roller or test tube . Excess blotting buffer		
	-	causes proteins to diffuse and cause bands to flow.		
	Incorrect buffer	Confirm the composition of the blotting buffer, and if it is dif-		
	composition	ferent or wrong, re-prepare fresh blotting buffer.		
	•	Carefully stack the filter papers, membrane and get to avoid		
Lineven er				
	Insufficient pretreatment			
dirty blotting	· ·			
	, ,	_		
	membrane.			
	The sandwich is not cen-			
		Place the sandwich in the center of the anode plate.		
Missing bands  Band flows  Uneven or dirty blotting	and the sandwich.  The gel was soaked in blotting solution for too long.  Blotting is not processed properly  Poor contact between the membrane and the gel.  Incorrect buffer composition  Air bubbles remain in the sandwich.  Insufficient pretreatment of the filter paper or membrane.	adjust the number of sheets to the same thickness referring to the number of filter sheets used.  Do not soak the gel before blotting for a long time (more than 5 minutes) in the blotting buffer.  In the case of high molecular weight proteins, the transfer efficiency can be improved by prolonging the blotting time and lowering the gel concentration of the electrophoresis gel. For low molecular weight proteins, this can be improved by increasing the amount of methanol added to the blotting buffer by about 1.5 to 2 times.  After stacking the filter paper, gel, and membrane, remove excess blotting solution and air bubbles by rolling over with using the blotting roller or test tube. Excess blotting buffer causes proteins to diffuse and cause bands to flow.  Confirm the composition of the blotting buffer, and if it is dif-		

Symptom	Cause	Countermeasure		
Uneven or dirty blotting	Filter paper is reused.	Filter paper is not reusable. Always use new filter paper.		
	The anode or cathode plate is soiled or substances are adhering to their surfaces	Clean the surface of the anode or cathode plates to remove dirt and adhering substances . If the contamination is severe, please contact the customer service center. (Please check the back cover.)		
	Wrong way to stack the sandwich	Check the order and number of layers of filter paper, and if incorrect, remake the sandwich using new filter paper.		
	Poor contact between the cathode plate and the sandwich.	Check the number and thickness of the filter paper. When using filter paper other than our product, use one with a thickness of 0.9 mm. If you use filter paper of other thickness, adjust the number of paper sheets to be the same thickness referring to the number of filter paper sheets.		
	Problems during the detection step	Contact the reagent manufacturer used for detection.		

#### 7 Maintenance

#### 7.1 Cleaning

#### 1.Power supply

If the surface becomes dirty, use a soft cloth soaked with mild detergent diluted with water and wipe gently. If the electrode connector gets dusty, remove it so as not to damage the connector.



When cleaning the main unit, remove the AC adapter and turn off the power switch. There is a risk of injury such as electric shock.

#### 2. AC adapter

If the surface becomes dirty, use a soft cloth soaked with mild detergent diluted with water and wipe gently. Do not use it until fully dried.



When cleaning the AC adapter, unplug it from the outlet. There is a risk of injury such as electric shock.

#### 3.The Cover

After use, wash with running water before the blotting buffer is dried. If the surface gets soiled, wash it with a soft sponge with a mild detergent. Rinse the cathode plate carefully with a soft sponge.

The cathode electrode plate may remain a white mark trace of filter paper, but please wash it gently with a soft sponge. If it is immediately after use, you can wash it by simply rubbing lightly.

In addition, please do not leave the device in water for long.

If dust is attached to the electrode plug, remove the plug not to damage it.

\* Do not press strongly the corner of the cathode plate. The internal spring mechanism may be deformed.



#### Warning

Remove the base and power supply part before cleaning the cover.

#### 4.The Base

After use, wash with running water before the blotting buffer is dried. If the surface gets soiled, wash it with a soft sponge with a mild detergent. Rinse the cathode plate carefully with a soft sponge.

If dust is attached to the electrode plug, remove the plug not to damage it



Remove the cover and power supply part before cleaning the base.

### 7.2 Inspection

Read this instruction manual and do inspection when using it for experiment after storage. If there is an abnormality when inspection, please contact us without using this device.

#### 1. Power supply



#### Warning

When inspecting the power supply section, remove the AC adapter and turn off the power switch. There is a risk of an accident such as getting an electric shock or injury.

#### 2. AC adapter

Please confirm that there is no peeling of the insulating coating, damage or deformation .



#### **Warning**

When checking the AC adapter, unplug it from the outlet.

There is a risk of an accident such as getting an electric shock or injury.

#### 3. Cover

Confirm that there is no damage, deformation or corrosion of the electrode plug. Also, check that the cathode plate is not dirty, press the center of the cathode plate, and that the vertical movement is smooth and that there is no difference in vertical movement between right and left side of the device.



#### Warning

Before inspecting the cover, remove the base.

#### 4. Base

Confirm that there is no damage, deformation, or corrosion of the electrode terminals.

When cleaning, make sure that the anode plate has no looseness and is firmly fixed.



#### Warning

Before inspecting the base, remove the cover.

### 7.3 Warranty

Please refer to the warranty attached to the product for the warranty and the warranty period.

# 7.4 Consumables

The followings are consumables. Please specify code number when ordering.

Application		Model	Product name	Code #
		WSE-4050	WSE-4050ClearBlot P+membrane (65x65mm 20pcs/box )	2322450
Blotting mem- brane	Membrane for Protein	WSE-4051	WSE-4051ClearBlot P+membrane (85x90mm、20pcs/box)	2322451
		WSE-4053	WSE-4053ClearBlot• P+membrane (260mmx3m)	2322453
	Pre-wet PVDF membrane	WSE-4055	QBlot kit (90 x85mm)	2322445
		CB-0 6 A	CB-06A filter paper ( 65x65mm, 400pcs)	2322437
Filter paper for blotting	Filter paper for blotting	CB-09A	CB-09A filter paper ( 85×90mm, 400pcs)	2392393
		CB-20A	CB-20A filter paper ( 20×20cm, 100pcs)	2392493
	3 Liquid system buffer for semi-dry blotting	AE-1460	EzBlot	2332600
Diatting buffer	High speed semi-dry blotting buffer	AE-1465	EzFastBlot	2332590
Blotting buffer	Semi-dry Fast Transfer buffer for High molecular weight proteins(200kDa <)	WSE-7210	EzFastBlot HMW	2332595
	Tris, Glycine buffer	WSE-7055	EzRun TG	2332323
	Non Protein for blocking buffer	AE-1475	EzBlock Chemi	2332615
Blocking buffer	BSA Blocking buffer	AE-1476	EzBlock BSA	2332616
	Casein Blocking buffer	AE-1477	EzBlock CAS	2332617
	Tris-Glysine buffer	WSE-7230	EzTBS	2332625
Wash buffer	Phoshate buffer saline solution	WSE-7430	EzPBS(-)	2332380
	10% Tween solution	WSE-7235	EzTween	2332626
	Coloring Substrate for HRP	AE-1490	EzWestBlue	2332630
	Luminol Substrate for HRP Detection	1110= =110	- W W 10	
Substrate for	(1 bottle type)	WSE-7110	EzWestLumiOne	2332632
detection	Luminol Substrate for HRP Detection	WSE-7120S	EzWestLumi Plus	2332637
	(High sensitive peroxidase substrate	WSE-7120L	EzWestLumi Plus	2332638
Reprobe reagent	Stripping reagent	WSE-7240	EzReprobe	2332530
Molecular weight	Colored molecular weight marker	AE-1450	EzStandardPrestainBlue	2332347
marker	Three colored protein ladder marker	WSE-7020	EzProtein Ladder	2332346
Sealing pack for gel & membrane	Clear film for gel and membrane Sealing		Pitatt Clear	2322438

# 8 Specifications

Model	PoweredBLOT 2M		
Model No.	WSE-4125		
System	Semi-dry blotting		
Valid blotting size	205 mm (W) x 100mm (D)		
Number of blotting gel	Mini size gel: 2		
Electrode Electrode size  Distance between electrodes  Polarity	205mm (W) x 100 mm (D) Min:3mm , Max:15mm Top: Cathode/ Bottom: Anode		
Power supply	STD mode: 12V Constant Voltage RAPID mode: 24V Constant Voltage Timer: 1-250min (display the remaining time) Power consumption: 26W Alarm: When output stops, time is up, errors are detected		
AC adapter Input Output	AC100V-240V ( $\pm 10\%$ ) 50Hz/60Hz ( $\pm 5\%$ ) DC24V 1.5A		
Safety measure	No load detection Short circuit detection		
Environment of usage	For indoor use only 5-40°C,5-70%RH, no condensation		
Condition	Portable equipment		
Dimension, weight of Main unit  AC adapter	246mm ( W) $\times$ 186mm ( D) $\times$ 114mm ( H) 2.1kg (Exclude protrusions and AC adapter when the power supply unit is attached) 36mm ( W) $\times$ 95mm ( D) $\times$ 31mm ( H) 0.14kg		
Components	WSE-4125 main body (The base, top cover, power supply) : 1 AC adapter : 1 Extension code : 1 Roller : 1 Instruction manual :1		



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