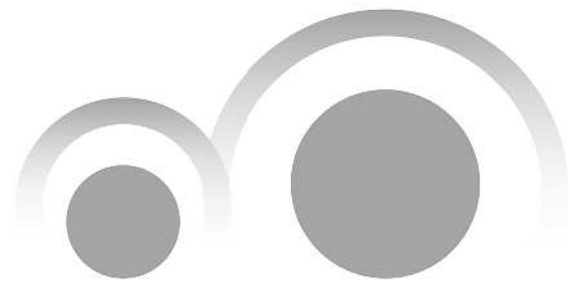
Biomate™

vBLOT4 electroblotting system for 4 gels

Cat. No. **VBLOT4G**



Instruction Manual

Contents

⋙ Introduction

After electrophoresis with **vPAGE/v2PAGE** - **vertical electrophoresis systems**, **vBLOT$_4$ electroblotting system** is used to blot proteins from gels to membranes by wet transfer (tank transfer).

vBLOT$_4$ can process up to 4 mini gels (8 x 9.6 cm) at the same time.

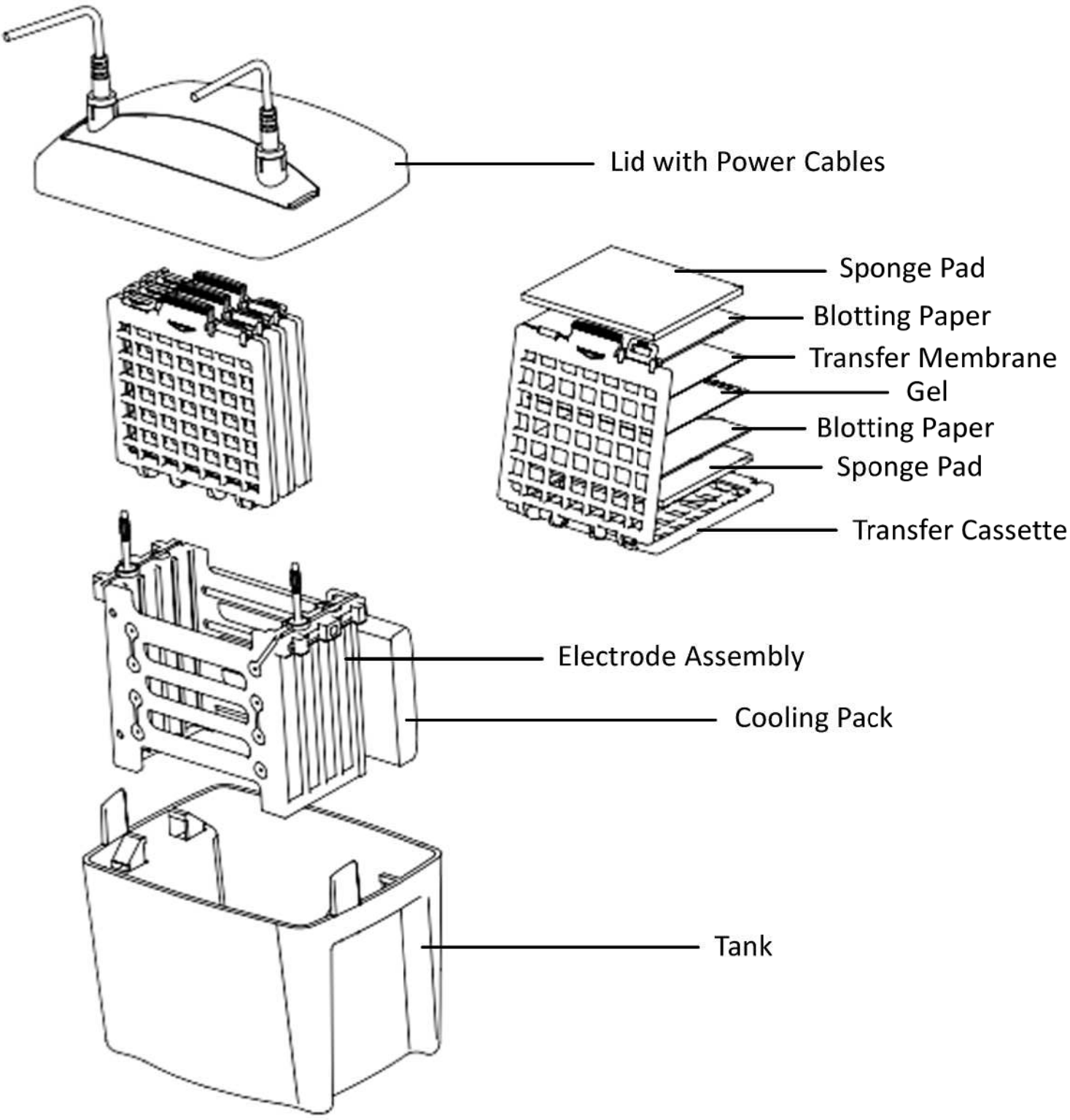
Besides protein, **vBLOT$_4$** can also transfer nucleic acids.

⋙ Packing List

Components of **vBLOT$_4$ electroblotting system for *4 gels***:

Item	Quantity	of Cat. No.
vBLOT$_4$ Lid with Power Cables	1	**V4LID**
vBLOT$_4$ Tank	1	**V4TANK**
vBLOT$_4$ Electrode Assembly	1	**VBLOT4E**
vBLOT$_4$ Transfer Cassette	4	**VBLOT4TC**
vBLOT$_4$ Sponge Pad	10	**VBLOT4SP**
vBLOT Cooling Pack	2	**VBLOT-CP**
Glass Plate Rack	1	**GPR**
Centrifuge Tube Rack	1	**CTR**

⋙ Specifications



⋙ Operating Instructions

⋙ Preparation before Electroblotting

- Store **Cooling Pack** at -20°C before and after use.
- Volumes with and without **Cooling Pack**:

w/ **Cooling Pack**	650 mL
w/o **Cooling Pack**	850 mL

- Prepare and pre-cool (at 4°C) transfer buffer[1]:

Tris	25 mM
Glycine	192 mM
Methanol	20%

[1] #BR140 (10X Western Transfer Buffer: 0.25M Tris-Base, 1.92M Glycine) is recommended.
For electroblotting with different transfer buffer, please check.

- Cut the transfer membrane and the blotting paper by the gel size.
- Activate the transfer membrane by slowly sinking into 20% (for NC[2]) or 100% (for PVDF[3]) methanol or ethanol, and agitating briefly to ensure it is fully wet (translucent).

[2] NC, the abbreviation of nitrocellulose.

[3] PVDF, the abbreviation of polyvinylidene fluoride.
#BMP30-300 (0.45µm PVDF Transfer Membrane, 30 cm x 3 m) is recommended.

Membrane should be handled carefully with gloves (ideally with rounded tweezers).

Do not allow transfer membranes to dry out during processing.

- Equilibrate (desalt) gels and soak blotting papers, sponge pads, and transfer membranes (after activation and rinsing with distilled water) in transfer buffer for 15-20 minutes (depending on the gel thickness).

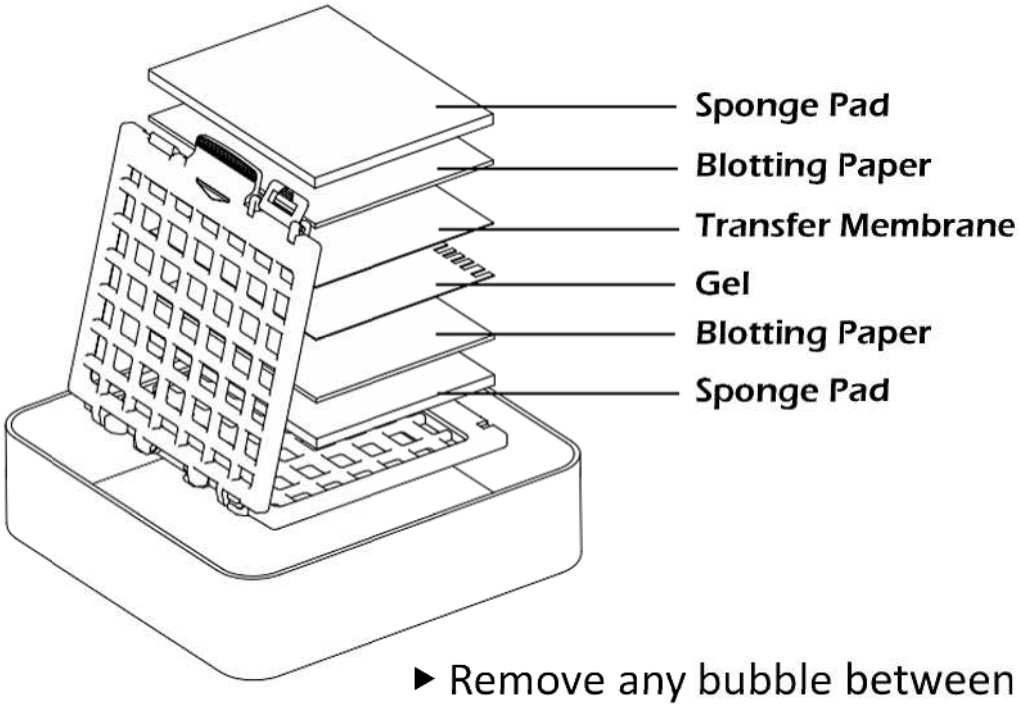
Excess heat may be produced during electroblotting if gels are not desalted.

Info. above is for reference. Please adjust depending on your applcation.

⋙ Setup and Run

❶ Place the opened **Transfer Cassette** on the bench with the black side down.

❷ Assemble as the image shows, and layer from bottom to top.

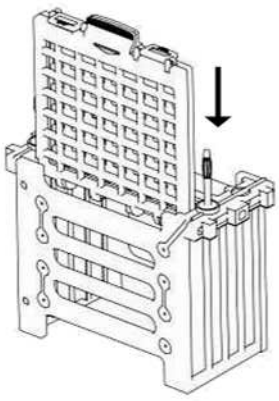


▶ Remove any bubble between layers by gently rolling it out with the roller[4], pipette or glass tube.

[4] #ROL (**vBLOT Roller**) is recommended.

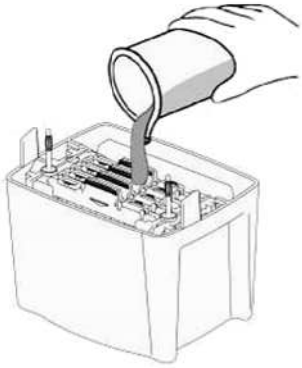
Bubbles between the gel and the transfer membrane would inhibit the blotting.

❸ Lock the cassette while being careful not to move any layer.



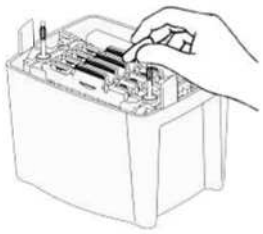
❹ Transfer the assembled cassette into **Electrode Assembly**, and place in the correct orientation (the black side of the transfer cassette aligned with the black half of the electrode assembly).

❺ Repeat all steps above to assemble another cassette.



❻ Place the frozen cooling pack into the tank, and then fill the tank with pre-cooled transfer buffer.

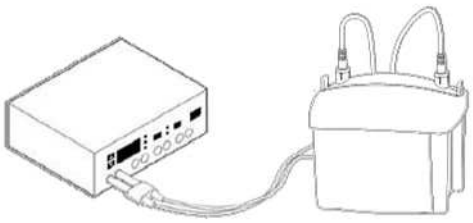
❼ Drop a stir bar into the buffer and set the stirring speed as high as possible to help distribute the temperature and ions evenly.



❽ Put the lid with power cables on, and connect to the power supply.

❾ Run at 100 volts with the constant current of 350 mA for 1 hour[5].

[5] The setting is for the transfer buffer described in Preparation before Electroblotting. For different buffer, please check Transfer Conditions with Different Buffers.



❿ When the run ends, remove the lid, disassemble the transfer cassette, and remove the membrane.

⓫ Now the transfer membrane is ready for Ponceau S[6] stain, Western Blot and further analysis.

[6] #BR590-500 (Ponceau S Staining Solution) is recommended.

⩥ Transfer Conditions with Different Buffers

	Transfer Type:		Standard	High Intensity
Transfer Buffer for SDS-PAGE	Tris	25 mM	30V constant 90mA	100V constant 350mA
	Glycine	192 mM		
	w/ or w/o	20% methanol 0.025-0.1% SDS		
Transfer Buffer for SDS-PAGE	Tris	48 mM	30V constant 90mA	100V constant 350mA
	Glycine	39 mM		
	w/ or w/o	20% methanol 0.025-0.1% SDS		
Transfer Buffer for SDS-PAGE	$NaHCO_3$	10 mM	30V constant 90mA	100V constant 350mA
	$NaCO_3$	3 mM		
	w/ or w/o	20% methanol 0.025-0.1% SDS		
	Transfer Time:		Overnight	1 Hour

Transfer Type:			Standard	High Intensity
DNA/RNA Transfer Buffer (TAE)	Tris	20 mM	30V constant 100mA	80V constant 500mA
	Sodium Acetate	10 mM		
	EDTA	0.5 mM		
	pH	7.8		
DNA/RNA Transfer Buffer (TBE)	Tris	50 mM	30V constant 90mA	80V constant 500mA
	Sodium Borate	50 mM		
	EDTA	1.0 mM		
	pH	8.3		
Transfer Buffer for Native Gel	Tris	25 mM	30V constant 90mA	100V constant 350mA
	Glycine	92 mM		
	pH	8.3		
Transfer Buffer for IEF and Acid-Urea Gel[7]	Acetic Acid	0.7%	30V constant 100mA	100V constant 350mA
Transfer Time:			**Overnight**	**1 Hour**

[7] To proceed the electroblotting of basic proteins from acidic gels, the positions of transfer membrane and gel must be switched while assembling the transfer cassette. In this scenario, proteins would move toward the cathode.

⋙ Troubleshooting

Problem	Possible Cause	Soultion
Poor Transfer (protein)	Transfer time is too short	Prolong the transfer time.
	Power issues	> Check the power supply and its functions (ex. current). > Check the settings. > Try the different transfer settings. > Check the integrity of platinum wires.
	Incorrect setup	Check the assembly of the transfer cassette, and the orientation of the cassette in the electrode assembly.
	Incorrect charge-to-mass ratio	Try the more acidic or basic transfer buffer.
	Protein precipitated	> Try the transfer buffer with SDS. > Decrease the use of methanol.

Problem	Possible Cause	Soultion
Poor Transfer (protein)	Methanol in transfer buffer	Decrease the use of methanol.
	Unsuitable transfer membrane	> Check the hydrophobicity/hydrophilicity of the protein sequence. PVDF membrane may work better for hydrophilic/polar/charged proteins. NC may work better for hydrophobic/non-polar proteins. > If working with small-sized proteins, try the membrane with the smaller pore size, or reduce the transfer time.
	Gel percentage is too high	Decrease the acrylamide percentage of the gel to increase the pore size, and thus the transfer efficiency.
Speckled or Swirled Bands	Contaminated transfer membrane	Minimize contact with membrane. Membrane should be handled carefully with gloves (ideally with rounded tweezers).
	Bubbles between the transfer membrane and the gel	Remove any bubble between layers by gently rolling it out with the roller, pipette or glass tube.
	Assembly of the transfer cassette is loose	> Try thicker blotting papers. > Sponge pads get compressed and become thinner after repeated uses. Replace with new sponge pads.
	High current	> Check the settings. > Use freshly prepared transfer buffer.
	Transfer membrane is not fully wet or dry out	Ensure the transfer membrane is fully wet during activation, and stay wet during the whole process.
	Unsuccessful electrophoresis	There are many factors leading to unsuccessful gel electrophoresis. To exclude this possible cause, confirm the result of electrophoresis by coomassie stain or silver stain.

Info. above is for reference. Please evaluate depending on your application.

⋙ Care and Maintenance

Major components of **vBLOT$_4$ electroblotting system** are made from polycarbonate, which is not compatible with **acetone**, **ketones**, **ethers**, and **aromatic/chlorinated hydrocarbons**.

Clean the electrode assembly, the transfer cassette and the tank with neutral detergent and warm water.

Be extremely careful with platinum wires when cleaning the electrode assembly.

Clean sponge pads with hot water, and then rinse with distilled water.

Defects caused by organic reagents are not covered by warranty.

Please contact us for concern of using reagents unmentioned.

⋙ Safety Information

- Please do not try to run without the lid.
- Power shall be off when the lid is opened.

Maximum Input	150 V 40 W
Maximum Operating Temperature	50°C

⋙ Ordering Information

Cat. No.	Product Description	Packing
V4LID	**vBLOT$_4$** Lid with Power Cables	1/pk
VPC	Power Cables	1/pk
V4TANK	**vBLOT$_4$** Tank	1/pk
VBLOT4E	**vBLOT$_4$** Electrode Assembly	1/pk
VBLOT4TC	**vBLOT$_4$** Transfer Cassette	2/pk
VBLOT4SP	**vBLOT$_4$** Sponge Pad	10/pk
VBLOT-CP	**vBLOT** Cooling Pack	1/pk
VBLOT4MINI	**vBLOT$_4$** Mini Module (Electrode Assembly x 1, Transfer Cassette x 4, Sponge Pad x 10, and Cooling Pack x 2)	1/pk
ROL	**vBLOT** Roller	1/pk

Cat. No.	Product Description	Packing
GPR	Glass Plate Rack	1/pk
CTR	Centrifuge Tube Rack	1/pk

▸ Related Products:

Cat. No.	Product Description
VBLOT2G	**vBLOT2** electroblotting system for ***2 gels***
VPAGE2G075	**vPAGE** - vertical electrophoresis system for ***2 gels, 0.75mm***
VPAGE2G100	**vPAGE** - vertical electrophoresis system for ***2 gels, 1mm***
VPAGE2G150	**vPAGE** - vertical electrophoresis system for ***2 gels, 1.5mm***
VPAGE4G075	**vPAGE** - vertical electrophoresis system for ***4 gels, 0.75mm***
VPAGE4G100	**vPAGE** - vertical electrophoresis system for ***4 gels, 1mm***
VPAGE4G150	**vPAGE** - vertical electrophoresis system for ***4 gels, 1.5mm***
V2PAGE2G075	**v2PAGE** - vertical electrophoresis system for ***2 gels, 0.75mm***
V2PAGE2G100	**v2PAGE** - vertical electrophoresis system for ***2 gels, 1mm***
V2PAGE2G150	**v2PAGE** - vertical electrophoresis system for ***2 gels, 1.5mm***
V2PAGE4G075	**v2PAGE** - vertical electrophoresis system for ***4 gels, 0.75mm***
V2PAGE4G100	**v2PAGE** - vertical electrophoresis system for ***4 gels, 1mm***
V2PAGE4G150	**v2PAGE** - vertical electrophoresis system for ***4 gels, 1.5mm***

⋙ Guarantee and Warranty

Biomate™ **vBLOT4 electroblotting system** is for research use only and guaranteed for twelve months from date of receipt.

The warranty does not cover defects caused by:

1) improper use,
2) organic reagents (check Care and Maintenance for further information), or
3) maintenance or repair by non-*Biomate*™/*Rainbow Biotech.* Staff.

Please contact the salesperson whom this product is purchased from for any question or concern.

⋙ Contact Information

▸ *Rainbow Biotechnology Co., LTD.*

www.rainbowbiotech.com.tw

biomate.com.tw

2F, No. 253, Sec. 4, Chung King North Road, Taipei 111, Taiwan

eMail: rainbow@rainbowbiotech.com.tw

Tel.: +886-2-28118200

Fax : +886-2-28118628