

Bio *ate*TM

vBLOT₂ electroblotting system **for 2 gels**

Cat. No. **VBL0T2G**



Instruction Manual

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» Introduction

After electrophoresis with **vPAGE/v²PAGE** - **vertical electrophoresis systems**, **vBLOT₂ electroblotting system** is used to blot proteins from gels to membranes by wet transfer (tank transfer).

vBLOT₂ can process up to 2 mini gels (7.5 x 10 cm) at the same time.

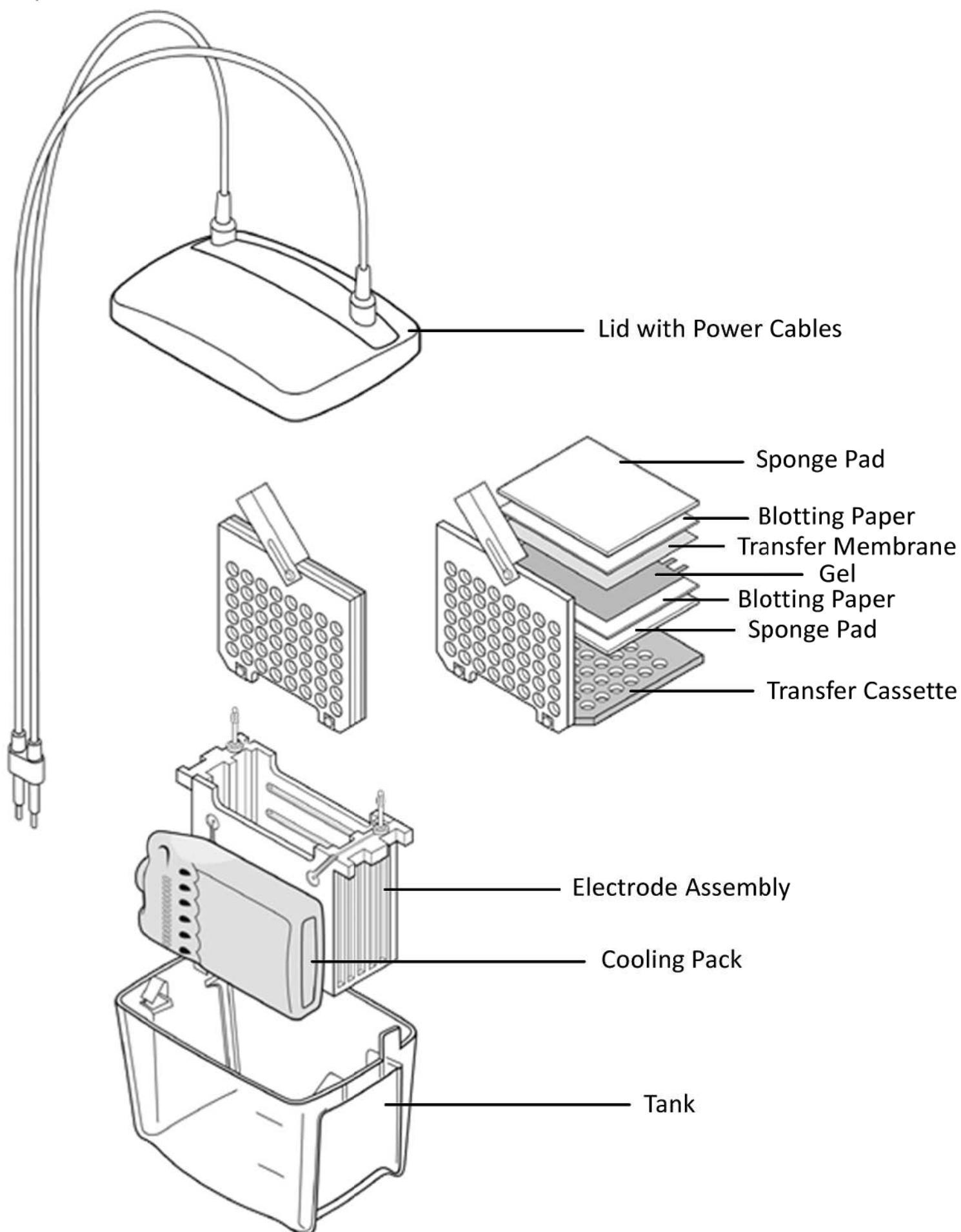
Besides protein, **vBLOT₂** can also transfer nucleic acids.

» Packing List

Components of **vBLOT₂ electroblotting system for 2 gels**:

Item	Quantity	of Cat. No.
vPAGE/v²PAGE/vBLOT₂ Lid with Power Cables	1	VLID
vPAGE/v²PAGE/vBLOT₂ Tank	1	VTANK
vBLOT₂ Electrode Assembly	1	VBLOT2E
vBLOT₂ Transfer Cassette	2	VBLOT2TC
vBLOT₂ Sponge Pad	5	VBLOT2SP
vBLOT Cooling Pack	2	VBLOT-CP

» 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 buffer¹:

¹ #BR140 (10X Western Transfer Buffer: 0.25M Tris-Base, 1.92M Glycine) is recommended.

Tris	25 mM
Glycine	192 mM
Methanol	20%

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 NC²) or 100% (for PVDF³) 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.

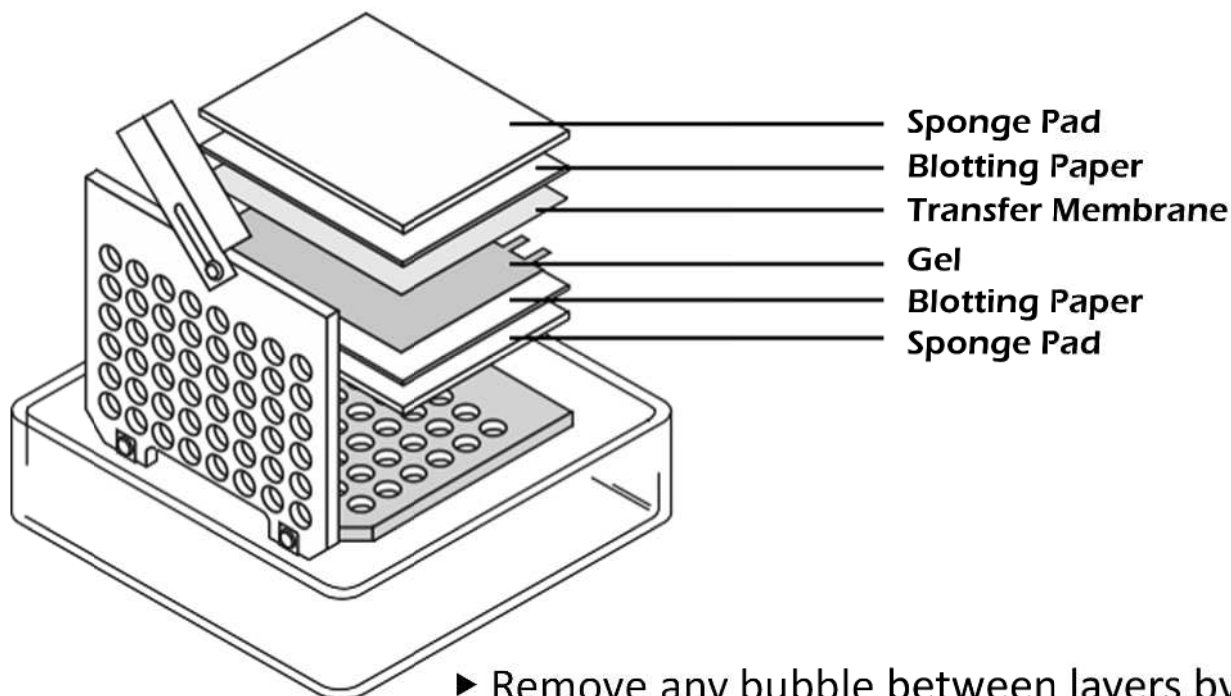
- ▶ Equilibrate (desalt) gels and soak blotting papers, sponge pads, and transfer membranes (after activation and rinsing with distilled water) in transfer buffer for 15-20 minutes (depending on the gel thickness).

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

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

» 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.

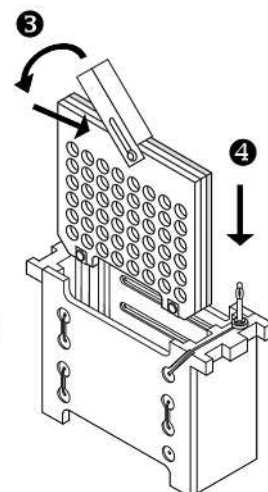


► Remove any bubble between layers by gently rolling it out with the roller⁴, pipette or glass tube.

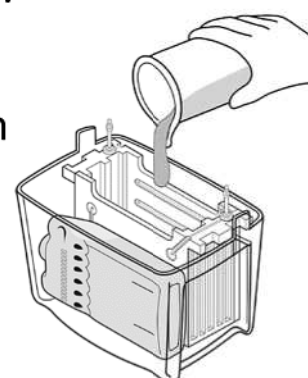
⁴ #ROL (vBLOT Roller) is recommended.

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

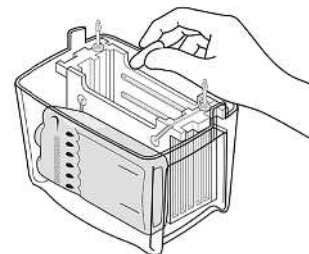
- ❸ Lock the cassette while being careful not to move any layer.
- ❹ Transfer the assembled cassette into **Electrode Assembly**, and place in the correct orientation (the black side of the transfer cassette aligned with the black half of the electrode assembly).
- ❺ Repeat all steps above to assemble another cassette.



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



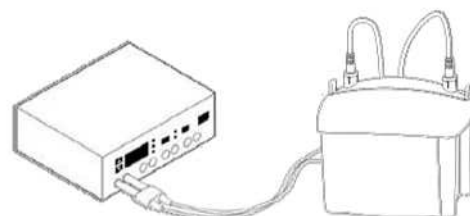
- ⑦ Drop a stir bar into the buffer and set the stirring speed as high as possible to help distribute the temperature and ions evenly.



- ⑧ Put the lid with power cables on, and connect to the power supply.

- ⑨ Run at 100 volts with the constant current of 350 mA for 1 hour⁵.

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



- ⑩ When the run ends, remove the lid, disassemble the transfer cassette, and remove the membrane.

- ⑪ Now the transfer membrane is ready for Ponceau S⁶ 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 **vBLOT₂ 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
VLID	vPAGE/v²PAGE/vBLOT₂ Lid with Power Cables	1/pk
VPC	Power Cables	1/pk
VTANK	vPAGE/v²PAGE/vBLOT₂ Tank	1/pk
VBLLOT2E	vBLOT₂ Electrode Assembly	1/pk
VBLLOT2TC	vBLOT₂ Transfer Cassette	2/pk
VBLLOT2SP	vBLOT₂ Sponge Pad	5/pk
VBLLOT-CP	vBLOT Cooling Pack	1/pk
VBLLOT2MINI	vBLOT₂ Mini Module (Electrode Assembly x 1, Transfer Cassette x 2, Sponge Pad x 5, 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
VBLOT4G	vBLOT₄ electroblotting system for 4 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™ vBLOT₂ 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

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