Biometra
An Analytik Jena Company

Vacu-Blot
Vacu-Blot System

Instruction Manual

Model	Part No.
Vacu-Blot System	**053-000, 053-090, 053-091**
Vacu-Blot	**053-100**

Please read these instructions carefully, before using this apparatus!

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Contents:

1 Intended Uses And Specifications

The **Vacu-Blot** enables the scientist to perform rapid and reliable transfer of nucleic acids (DNA and RNA) from agarose gels onto membranes. This technique is superior to any other way of nucleic acid transfer because of its speed. Compared with the usual capillary transfer the vacuum-blotting demonstrates an enhanced detection limit of nucleic acids[1]. The size of the Vacu-Blot is adequate for medium-sized up to large-sized agarose gels (max. 20 x 20 cm) as routinely used in molecular biology laboratories.

The efficiency of transfer of nucleic acids mainly depends on the vacuum. The filter plate holder has therefore been optimised to ensure an homogeneous vacuum over the entire filter area. The size of the collecting tank has been designed in order to guarantee an appropriate vacuum but is also large enough for collecting the entire flow through during a 1 h transfer. By using a vacuum pump (included in the Vacu-Blot System), which can be regulated, the transfer of nucleic acids becomes a simple and reproducible procedure with a superior degree of efficiency[1]. A simple water jet pump is not adequate for the vacu-blot method.

The rubber mats, which are delivered together with the apparatus, are used for sealing. In the centre of one mat a piece is cut out, which is slightly smaller than the agarose gel. The transfer membrane, which lies on the filter plate, is covered by the rubber frame and the agarose gel placed above the empty central hole of the frame. The cover plate (filled with transfer buffer) presses the rubber mat onto the filter plate. Therefore, after applying vacuum, the entire surface of the filter plate is sealed by the rubber frame with the exception of the central hole. The buffer is forced to migrate through the agarose gel and to transport the nucleic acid fragments onto the membrane.

The collecting tank and the cover plate are manufactured from durable acrylic, which withstands most reagents.

[1] Olszewska, E. and Jones, K. (1988). Vacuum blotting enhances nucleic acid transfer. TIG 4: 92-94.

2 Systems and Accessories Available

Vacu-Blot (Order No. 053-100)

- Transfer unit, consisting of
  Collecting tank (volume: 3400 ml)
  Porous filter plate
  Filter plate holder (23.7 x 23.7 cm)
  Cover plate with buffer reservoir (volume: 3000 ml)
- 9 clips (3 x Order No. 010-007)
- 3 Rubber masks (27.5 x 27.5 cm) (Order No. 053-002)
- 1 Tube adapter (Vacuum connector) for the collecting tank
- 1 Vacuum tube (Order No. 049-001)
- 1 Instruction Manual

Vacu-Blot System	**230V**	**(Order No. 053-000)**
	115V, 60Hz	**(Order No. 053-090)**
	100V, 50/60Hz	**(Order No. 053-091)**

- Vacu-Blot (complete transfer unit with accessories)
- Vacuum pump MP86 with manometer and adjustable vacuum gauge

3 Ordering Information

Item:	Order No.:
Vacu-Blot System with vacuum pump (20 x 20 cm gel size), 230 V	053-000
Vacu-Blot System with vacuum pump (see above), 115 V, 60 Hz	053-090
Vacu-Blot System with vacuum pump (see above), 100 V, 50/60 Hz	053-091
Vacu-Blot without vacuum pump	053-100
Vacuum pump MP86 with manometer and adjustable vacuum gauge (max. delivery 6 l/min, end vacuum 100 mbar), 230 V	049-000
Vacuum pump MP86 with manometer and adjustable vacuum gauge (max. delivery 6 l/min, end vacuum 100 mbar), 115 V, 60 Hz	049-090
Vacuum pump MP86 with manometer and adjustable vacuum gauge (max. delivery 6 l/min, end vacuum 100 mbar), 100 V, 50/60 Hz	049-091
Accessories:	
Porous filter plate for Vacu-Blot/System	053-004
Rubber mat (3 pieces) for Vacu-Blot/System	053-002
Clips (3 pieces)	010-007

Paper:		
Whatman 3MM Chr	100 pieces, 46 cm x 57 cm (thickness: 0.34 mm)	3030917
Whatman 3MM Chr	100 pieces, 58 cm x 68 cm (thickness: 0.34 mm)	3030931

Protran® and Optitran® Blotting Membranes:	
Whatman Protran-BA83, 0,20 µm pore size, 30 cm x 3 m, 1 roll	B10 401 396
Whatman Protran-BA85, 0,45 µm pore size, 30 cm x 3 m, 1 roll	B10 401 196
Whatman Protran-BA85, 0,45 µm pore size, 30 cm x 60 cm, 5 sheets/pack	B10 401 180
Whatman Optitran-BA-S 85, 0,45 µm pore size, 30 cm x 3 m, 1 roll	B10 439 196

4 Safety and Operating Informations

Caution!
Do **not** use **organic solvents** or solvents with chaotropic ions for cleaning.

Solvent resistance:	Use **neither** organic solvents (e.g.: acetone, chloroform), **nor** >30% ethanol; Use **no** chaotropic ions (e.g. urea, guanidine hydrochloride)	
Cleaning:	A detergent solution 0.1 N NaOH solution a dilute alcohol solution (≤ 30 %)	or or
Vacuum:	Maximum 500 mbar less than atmospheric pressure	
Max. gel size	20 x 20 cm	

5 Set Up

5.1 Unpack and Check

Unpack and carefully examine the electrophoresis unit. Report any damage to BIOMETRA. Do not attempt to operate this device if physical damage is present. Save all packing material if damage is found.

Do not attempt to operate this device if physical damage is present.

⚠ **!! Attention !!** ⚠
Please fill out and send back the warranty registration card. This is important for you to claim to full warranty.

6 Location

Place the chamber in proximity to the vacuum pump with which it is to be connected. Be sure to place the chamber in a safe, dry location away from the edge of the working surface.

7 Assembling the Transfer Unit

⚠ ***Prior to use, ensure that the filter plate and the filter plate holder are not clogged.***

⚠ ***Before inserting a membrane into the Vacu-Blot, please read the information about pre-treatment of the desired filter material given by the manufacturer.***

1. Screw the tube adapter into the housing of the collecting tank. The vacuum pump can either be connected directly to the Vacu Blot or indirectly via an intermediate wash bottle. The use of a wash bottle is advisable when working with radioactive substances or very concentrated saline solutions (e.g.: 10 x SSC).

2. Place the filter plate holder into the collecting tank and place the porous filter plate on it.

3. Place a piece of Whatman 3MM filter paper, which has been cut to the size of the filter plate and equilibrated in the transfer buffer (e.g.: 2xSSC-buffer), on the filter plate.

   ⚠ ***Note:*** *Eliminate all air bubbles between the filter paper and the filter plate by rolling a glass pipette or a similar object across the filter paper.*

4. The membrane, which has been pre-cut to fit your sample needs and equilibrated in buffer solution, is laid on the filter paper.

   ⚠ ***Note:*** *Eliminate all air bubbles between the membrane and filter paper by rolling a glass pipette or a similar object across the membrane.*

5. Place a wet rubber mat, from which the centre has been cut out (see note), on the filter plate. This rubber frame covers the entire surface of the filter plate with the exception of the cut out centre.

   ⚠ ***Note:*** *The size of the cutting in the rubber mat should be slightly smaller than the agarose gel (maximum size of agarose gel 20 x 20 cm). Eliminate all air bubbles between the rubber mat and the membrane by rolling a glass pipette or a similar object across the rubber mat.*

6. Place the agarose gel on the central hole of the rubber frame. There should be about 5 - 10 mm of the agarose gel on each side lying on the rubber mat. The sample slots must lie on the rubber mat otherwise inhomogenity of the vacuum around the slots could occur during transfer.

   ⚠ ***Note:*** *Eliminate all air bubbles between the agarose gel, the membrane and the rubber frame by rolling a glass pipette or a similar object across the gel.*

7. Place the cover plate with the buffer reservoir on the filter plate so that it corresponds to the orientation of the guide pins.

8. Tighten both parts of the Vacu Blot by using the clips.

9. Apply vacuum to the transfer unit before pouring transfer buffer into the upper reservoir. Otherwise your agarose gel might float up.

10. Transfer time of nucleic acid fragments from an 0.8 % concentrated agarose gel to the membrane is about 1 h to 2 h (when applying a vacuum of 50 to 100 mbar below ambient). These values are only guideline for transferring nucleic acids from 0.5 kb - 10 kb.

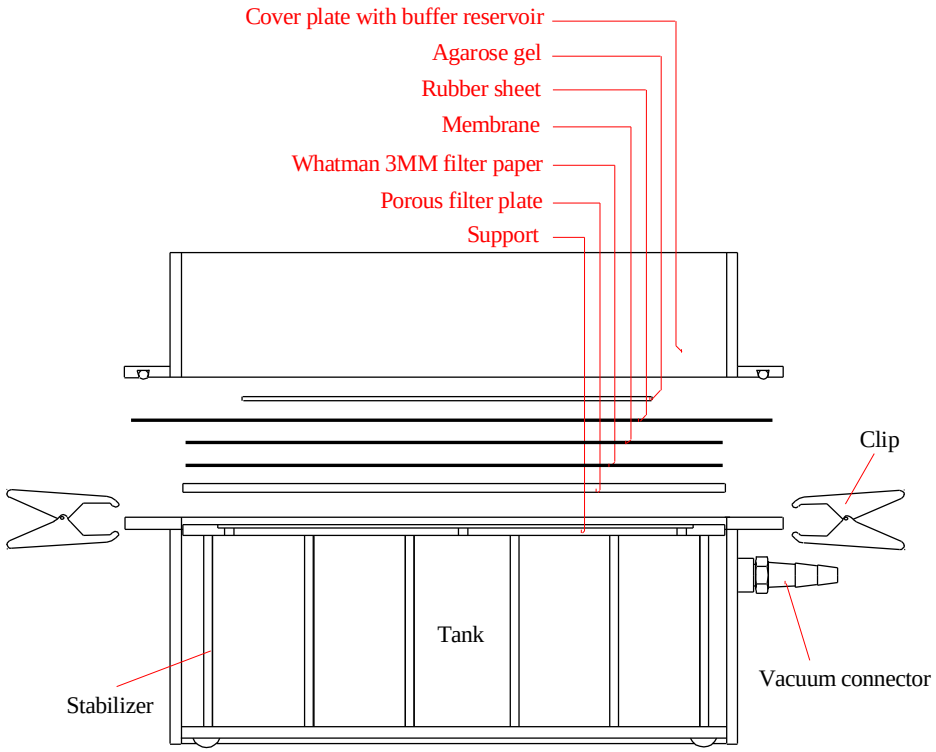

Fig. 1: Set up of a vacuum blotting sandwich

8 Disassembling the unit

1. First interrupt the vacuum before opening the clips. When using the vacuum pump delivered with the system (Order-No.: 053-000/090/091) you can apply ambient pressure by opening the flow valve.

2. Remove the transfer buffer from the upper buffer reservoir. Afterwards the apparatus can be disassembled in reverse order to assembling.

3. The membrane can be further processed as usually, e.g. by UV-light crosslinking or by baking at 80°C.

4. Please rinse all parts of the Vacu Blot after use with deionised water. Pay attention to the guide lines for cleaning given in chapter **"4.0 Safety and Operating Informations"**.

9 Application Information

9.1 Southern Blot with the Vacu-Blot and Vacu-Blot System

Prior to transferring the DNA should be pre-treated in order to increase the efficiency of the vacu-blotting. The agarose gel should be handled in incubation trays during the different pre-treatment steps, but never in the apparatus.

Depurination	0.25 M HCl	2 x 15 min
Denaturation	0.5 M NaOH 1.5 M NaCl	2 x 15 min
Neutralisation	3.0 M NaCl 0.5 M Tris, pH 7.4	3 x10 min
Equilibration in Transfer Buffer	0.3 M NaCl 0.03 M Na_3Citrat	30 - 60 min

or:

Depurination	0.25 M HCl	2 x 15 min
Denaturation	0.4 M NaOH 1.5 M NaCl	2 x 15 min
Neutralisation	1.5 M NaCl 1 M Tris, pH 7.4	3 x10 min
Equilibration in Transfer Buffer	2 X SSC	30 - 60 min

Note:
RNA and DNA only bind to nitrocellulose in solutions with a high ionic strength. When using SSC the membrane should be soaked in 20 x SSC before the transfer.

Some nylon membranes bind DNA even at basic pH-values. Therefore a separate step for neutralisation is not necessary. See the following scheme.

Depurination	0.25 M HCl	2 x 15 min
Denaturation	0.5 M NaOH 1.5 M NaCl	2 x 15 min
Equilibration in Transfer Buffer	0.5 M NaOH 1.5 M NaCl	30 - 60 min

Alternatively 2 X SSC can be used as transfer buffer.

9.2 Northern Blot with the Vacu Blot and Vacu-Blot System

To support an efficient hybridization of the probe, the RNA is denatured before and during electrophoresis:

Denaturation	50 % (v/v) Formamide (deionised) 6 % (v/v) Formaldehyde 1 x SSC	15 min, 65°C

or:

Denaturation	50 % (v/v) DMSO 1 mM Glyoxal 12,5 mM Sodium phosphate 1 x SSC	15 min, 50°C

Between the denaturation and the transfer the samples are to be stored on ice.

Note:
DMSO attacks nitrocellulose membranes. Do not use DMSO together with nitrocellulose membranes and extend the incubation time to 1 h.

In case of huge RNA molecules a denaturing step at high pH (basic) could improve transfer efficiency.

Denaturation	0.05 M NaOH 0.01 M NaCl	30 min
Neutralisation	0.1 M Tris, pH 7.5	30 min

10 Service

Should you have any problems with this unit, please contact our service department or your local Biometra dealer:

Biometra GmbH
Service Department
Rudolf-Wissell-Straße 14 - 16
D-37079 Goettingen
Phone: +49 (0)5 51 50 68 6 - 10 or 12
Fax: +49 (0)5 51 50 68 6 -11
e-mail: Service@biometra.com

If you would like to send the unit back to us, please read the following return instructions.

Instructions for return shipment

Return only defective devices. For technical problems which are not definitively recognisable as device faults please contact the Technical Service Department at Biometra (Tel.: +49 (0)5 51-50 88 1-10 or -12, Fax: +49 (0)5 51-50 88 1-11, e-mail: Service@biometra.com).

Please contact our service department for providing a return authorization number (RAN). This number has to be applied clearly visible to the outer box. Returns without the RAN will be not be accepted!

Important:

Carefully clean all parts of the instrument from residues, and of biologically dangerous, chemical or radioactive contaminants. If an instrument is contaminated, Biometra will be forced to refuse to accept the device. The sender of the repair order will be held liable for possible damages and losses resulting from insufficient decontamination of the device.

Please prepare written confirmation (use the “Equipment Decontamination Declaration” following on the next page) that the device is free of biologically dangerous, chemical or radioactive contaminants. This confirmation must be attached to the outside of the packaging.

Use the original packing or a similarly robust packing when returning the device. If not available, contact Biometra or your local distributor.

Label the outside of the box with “CAUTION! SENSITIVE INSTRUMENT!” and the **RAN** number sticker. Attach the Decontamination Declararion!

Please enclose a note which contains the following:
a) Sender’s name and address,
b) Name of a contact person for further inquiries with telephone number.
c) Precise description of the fault, which also reveals during which procedures the fault occurred, if possible.

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11 Equipment Decontamination Certificate

To enable us to comply with german law (i.e. §71 StrlSchV, §17 GefStoffV and §19 ChemG) and to avoid exposure to hazardous materials during handling or repair, please complete this form, prior to the equipment leaving your laboratory.

COMPANY / INSTITUTE _____

ADDRESS _____

PHONE NO _____ FAX NO_____

E-MAIL _____

EQUIPMENT	Model	Serial No
	_____	_____
	_____	_____
	_____	_____
	_____	_____

If on loan / evaluation Start Date: _____ Finish Date _____

Hazardous materials used with this equipment:

_____

_____

_____

Method of cleaning / decontamination:

_____

_____

_____

The equipment has been cleaned and decontaminated:

NAME _____ POSITION _____
(HEAD OF DIV./ DEP./ INSTITUTE / COMPANY)

SIGNED _____ DATE _____

PLEASE RETURN THIS FORM TO BIOMETRA GMBH OR YOUR LOCAL BIOMETRA DISTRIBUTOR TOGETHER WITH THE EQUIPMENT.
PLEASE ATTACH THIS CERTIFICATE OUTSIDE THE PACKAGING. INSTRUMENTS WITHOUT THIS CERTIFICATE ATTACHED WILL BE RETURNED TO SENDER.

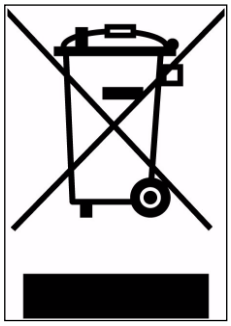
General Information for Decontamination:

Please contact your responsible health & safety officer for details.

Use of radioactive substances:
Please contact your responsible person for details.

Use of genetically change organism or parts of those:
Please contact your responsible person for details.

12 Note for Disposal of Electric/Electronic Waste



This symbol (the crossed-out wheelie bin) means, that this product should be brought to the return systems and/or separate systems available to end-users according to yours country regulations, when this product has reached the end of its lifetime!

For details, please contact your local distributor!

This symbol applies only to the countries within the EEA*.

*EEA = European Economics Area, comprising all EU-members plus Norway, Iceland and Liechtenstein.