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Operating Manual

Biometra Fastblot B43 & B44
Semi-Dry Blotter

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For a proper and safe use of this product follow the instructions. Keep the operating manual for future reference.

General Information http://www.analytik-jena.com

Documentation Number 34-2201-102-23

Edition A (10/2023)

Technical Documentation Analytik Jena GmbH+Co. KG

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1 Notes on this operating manual

Content

This user manual describes the following device models:
- Biometra Fastblot B43
- Biometra Fastblot B44

In this manual, these models are collectively referred to as the **device**. Any differences between the models are explained in the relevant section.

The device is intended to be operated by qualified specialist personnel under observance of the operating manual.

The operating manual provides information about the design and operation of the device and provides operating personnel with the necessary know-how for safe handling of the device and its components. Furthermore, the operating manual includes information on the maintenance and servicing of the device as well as information on potential causes of malfunctions and their correction.

Conventions

Instructions for actions occurring in chronological order are numbered and combined into action units.

Warnings are indicated by a warning triangle and a signal word. The type, source and consequences of the hazard are stated together with notes on preventing the hazard.

Elements of the control and analysis program are indicated as follows:
- Program terms are in bold (e.g., the **System** menu).
- Menu items are separated by vertical lines (e.g., **System** | **Device**).

Symbols and signal words used in this manual

The user manual uses the following symbols and signal words to indicate hazards or instructions. These warnings are always placed before an action.

WARNING

Indicates a potentially hazardous situation which can cause death or very serious (possibly permanent) injury.

CAUTION

Indicates a potentially hazardous situation which can cause slight or minor injuries.

NOTICE

Provides information on potential material or environmental damage.

2 Intended use

The Biometra Fastblot is an electroblotter for transferring proteins from polyacrylamide gels to a carrier membrane.

The device permits the use of discontinuous buffer systems for either carefully blotting smaller proteins, or for uniform transfer of the most diverse proteins.

The cooling water ports on the anode of the Fastblot B43 model allow generated heat to be dissipated during the blotting process.

The device can also blot nucleic acids. Transferring nucleic acids using the vacuum method is recommended, however.

The device is suitable for a maximum gel size of 15.5 cm x 19.5 cm.

3 Safety

For your own safety and to ensure error-free and safe operation of the device, please read this chapter carefully before commissioning.

Follow all safety instructions described in this manual.

3.1 Safety labeling on the device

Warning and mandatory action labels have been attached to the device and must always be observed. Damaged or missing warning and mandatory action labels can cause incorrect actions leading to personal injury or material damage.

- Do not remove the warning and mandatory action signs.
- Replace damaged signs.

The following warning and mandatory action signs are used:

Warning/mandatory sign	Meaning
	General warning sign, observe the operating manual!
	Fragile device parts!
	Danger of electric shock!

3.2 Requirements for the operating personnel

The device must only be operated by qualified specialist personnel instructed in the use of the device. The operating personnel must meet the following requirements:

- Only operate the device after reading the operating manual.
- Know and avoid dangers when working with the device.
- Wear personal protective equipment such as protective gloves, lab coat and safety goggles.
- Training by Analytik Jena is recommended.

The operator of the device is responsible for compliance with safety and occupational health regulations. The operator must meet the following requirements:

- Provide information about national regulations on work safety and accident prevention and observe them during operation of the device.
- Instruct the operating personnel in the safe operation of the device. In doing so, also convey the contents of the manuals for the device system.

3.3 Safety instructions for transport and commissioning

Transport

There is a risk of injury when lifting and carrying, especially from unsecured parts.

- Empty the device, close the cover and, on the Biometra Fastblot B43 model, remove any connected hoses. Secure all loose parts, with adhesive tape, for example.
- Only transport the device in its original packaging. Insert all transport locks.

Ambient conditions during commissioning

The device is dangerous if it is installed in an unsuitable environment.

- Place the device on a dry, flat surface.

Electrical conditions

The device may be dangerous if the conditions for the electrical connection are not met.

- Only protection class 2 power supply devices are suitable for the blotting systems. Observe the specifications in the operating manual of the power supply device used!
- Check the electrical requirements of the device before connecting it to a power supply device.
- Never place the device on top of a power supply device.
- Only use the supplied cables for connection to a power supply device.
- Disconnect the cable connection between the device and the power supply device before storage.

3.4 Safety instructions for operation

Electrical hazard

Lethal voltages may occur in the device.

- Before each start-up, make sure that the device and its safety devices are in proper working condition.
- In case of faulty electrical components, switch off the power supply device immediately, disconnect it from electrical power, and disconnect the connection between the device and the power supply device.
- Do not remove or bypass any protective devices such as the housing.
- Before opening the cover, switch off the power supply device and disconnect the power connection to the device.
- Do not use the device in environments with extreme humidity (>95 %), or in locations in which condensation occurs.

Hazard from substances

The device can be used to handle hazardous substances. The operator is responsible for the safe handling of these substances.

- If the device has been contaminated with hazardous substances, decontaminate it as described in the operating manual. Use other methods only after consultation with Analytik Jena.

3.5 Safety instructions for maintenance and cleaning

There is a risk of electric shock if contact is made with live components, which may lead to serious injury.

Unauthorized servicing can lead to maladjustment or damage of the device and its system components.

- Only carry out the maintenance actions listed in the operating manual.

- Before maintenance and cleaning, switch off the power supply device and disconnect the power connection between the device and the power supply device. Only work on the electrically live device if the operating manual explicitly requires it.
- Only use original spare parts. These have been tested and ensure safe operation.
- After maintenance, ensure that all safety devices are fully functional again.
- Clean the device with a damp, non-dripping cloth. Do not use alcohols with a concentration ≥ 10 %. Do not use organic solvents, abrasive cleaners or bleach.

4 Design

Device components

The device has the following elements:

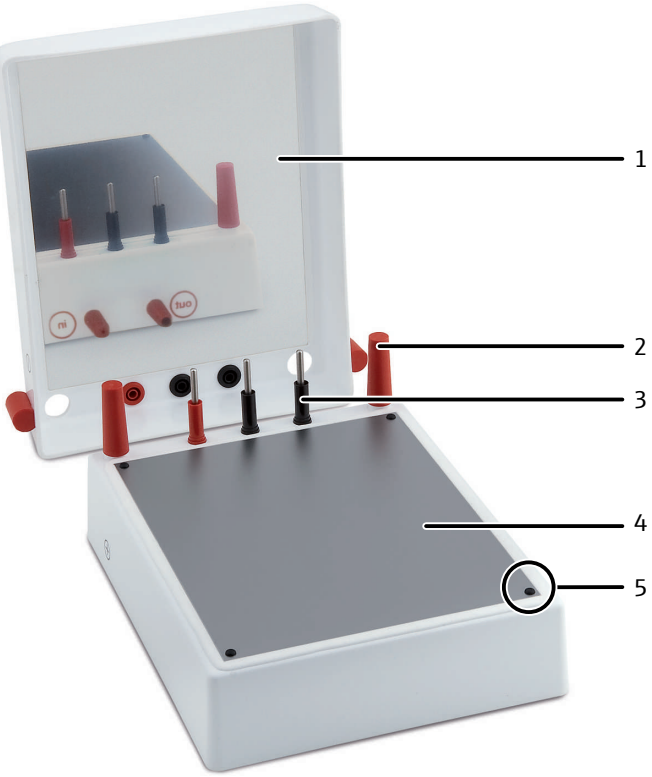

Fig. 1 Components of the device

1 Cathode (special stainless steel)
2 Guide pins for cover placement
3 Safety connections for the cover
4 Anode (platinum-plated titanium)
5 Spacer

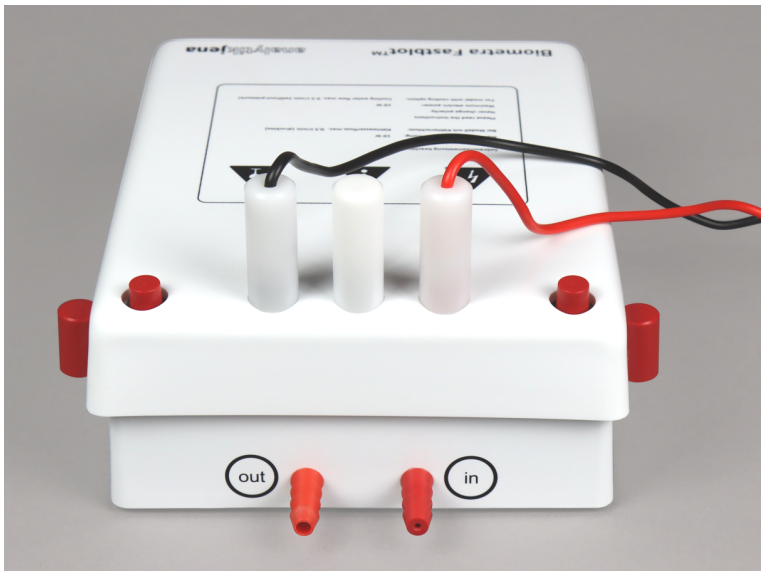

Fig. 2 Connections for continuous-flow cooling on the rear of the Fastblot B43

Type plate

The type plate is located on the bottom of the device and includes the following information:

- Manufacturer and address
- Device type and model
- Year of manufacture
- Country of manufacture
- Electrical connection data
- Serial number
- Conformity and test sign
- Disposal instructions (Do not dispose of as domestic waste!)

5 Installation and commissioning

5.1 Spatial requirements

The spatial requirements for the models are listed in the following table.

Additional space is required next to the device for the power supply device.

Model	Dimensions
Biometra Fastblot B43	26 cm x 22 cm x 11 cm (LxWxH)
Biometra Fastblot B44	24 cm x 22 cm x 11 cm (LxWxH)

⚠ CAUTION! Never place the blotter on top of the power supply device! Escaping buffer fluid can enter the power supply device, leading to electric shocks.

5.2 Power supply

Independent of the specifications of the power supply device used, the following maximum threshold values for use of the device apply:

Max. voltage	50 V
Max. current per cm^2 of gel	5 mA
Max. power	10 W For max. 30 min. operating time: 20 W Biometra Fastblot B43: For max. 30 min. operating time and with cooling at 12 °C: 30 W

5.3 Installation

NOTICE

Keep the original packaging

Transport damage can only be avoided if the device is transported in its original packaging.

- Keep the original packaging for transport, e.g., in case the device must be returned to the manufacturer for repair.

- Remove the device from the transport packaging.
- Verify that the delivery is complete. Check all components of the device for transport damage.
  - If the delivery is incomplete or transport damage has occurred, contact Analytik Jena.
  - In case of a return shipment, observe the information in the corresponding chapter of this operating manual.
- Place the device on a dry, flat surface.

  ✓ The device has been installed.

6 Operation

CAUTION

Danger of skin injury

Wear suitable protective clothing when operating the device and handling the chemicals used.

6.1 Tips for blotting efficiency

The following sections show optimization option for blotting proteins with the Biometra Fastblot B43 and Biometra Fastblot B44 models.

NOTICE! Information on blotting nucleic acids is provided in a separate chapter.

Checking transfer efficiency

You can check the efficiency of the transfer by using standard proteins to which Coomassie Brilliant Blue is covalently bound.

After the blotting process, you can dye the gel with Coomassie Brilliant Blue to check the completeness of the transfer.

Transfer conditions

The transfer conditions (time, current) should be optimized for each protein. The following illustration outlines the transfer times and rates of some proteins. Rough estimates for transfer times can be made with the aid of this illustration.

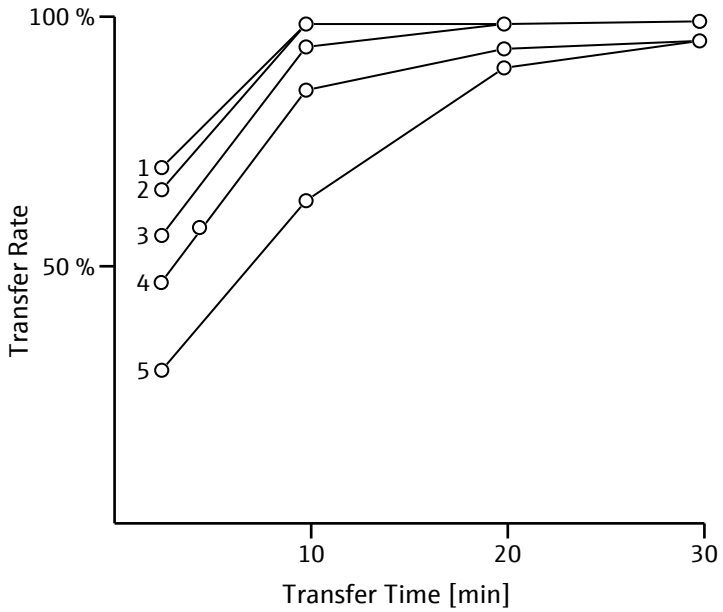

Fig. 3 Electrophoretic transfer of proteins. SDS-PAGE, acrylamide concentration: 10 %; nitrocellulose blotting membrane, pore size: 0,45 µm; current: 5 mA/cm² gel surface; gel thickness: 1.0 mm; transfer buffer: Tris/glycine/SDS

1 Carbonic anhydrase (molecular weight: 29 kDa)
2 Albumin (molecular weight: 40 kDa)
3 Bovine serum albumin (molecular weight: 67 kDa)
4 β-galactosidase (molecular weight: 116 kDa)
5 Myosin (molecular weight: 205 kDa)

The transfer time is also affected by the gel thickness and the acrylamide concentration.

If the selected transfer time is too long, the protein may pass the membrane and be lost. This can be checked with the aid of a second membrane under the actual blotting membrane, which is also tested for proteins after the transfer.

Using continuous-flow cooling

In the following cases, the use of the Fastblot B43 model with cooling system is recommended:

- Transferring proteins with a high molecular weight > 100 kDa
  The blotting process can take up to 30 minutes for higher molecular weights. A longer blotting process increases the risk of the device overheating, which can lead to damage to the device and the sample.
- Transferring native proteins or enzymes

Effect of methanol

The use of methanol has the following effects on the blotting process:

- Improved protein binding to nitrocellulose
- Inhibits gel swelling
- Potentially poorer blotting of the proteins (reduction of protein solubility)

Effect of SDS

The use of SDS has the following effects on the blotting process:

- Inhibits protein binding to the membrane
- Even charge of the proteins
- Potential changes to the antigen properties of a protein

6.2 Tips for nucleic acid blotting

You can also use the blotter for the electrotransfer of nucleic acids on agarose gels to a membrane.

The device is primarily intended for protein blotting. Observe the tips and information in this chapter when blotting for this reason.

NOTICE! Use of the vacuum blotting method (DNA/RNA) or the capillary blotting method (RNA) is better suited to the transfer of nucleic acids.

Pretreatment

To increase blotting efficiency, samples with nucleic acids should be pretreated before transfer:

Work step	Substance	Time interval
Depurination	0.25 M HCl	7 min
Denaturation	0.50 M NaOH	15 min
	1.50 M NaCl	
Neutralization	3.00 M NaCl	15 min
	0.50 M tris (pH 7.4)	

The blotting sandwich is set up corresponding to the protein blotting process. Make the following changes to the process:

Buffer

- Transfer buffer: 1 x TAE or 1 x TBE

Blotting sandwich

- Blotting paper: 10 pcs. Whatmann 3MM (soaked with transfer buffer) each above and below the blotting sandwich
- Membrane: Do not use nitrocellulose membranes for nucleic acid blotting! Nitrocellulose is not stable in basic solutions. You can use nylon membranes, for example.
- Membrane position: Place the membrane on the anode side of the gel.

Blotting process

- Transfer time: No longer than 30 minutes

6.3 Blotting process

NOTICE

The device is primarily intended for protein blotting. Observe the tips and information in the "Tips for nucleic acid blotting" chapter when blotting nucleic acids for this reason.

6.3.1 Preparing buffer solution

NOTICE

Do not use a buffer with pH < 3 or pH > 10!

Solutions for continuous transfer buffer systems

Transfer buffer, pH 8.3

- 25 mM tris base
- 150 mM glycine
- 10 % methanol

	Optionally: Dilute the transfer buffer with distilled water (single or double volume).
Electrophoresis buffer (Laemmli system)	■ 25 mM tris base ■ 192 mM glycine ■ 0.1 % SDS (sodium dodecyl sulfate) Dilute the electrophoresis buffer for blotting with one or two parts distilled water and add 10–20 % methanol.
Towbin buffer	■ 50 mM tris ■ 9 mM glycine ■ pH 7.8–8,4

Solutions for discontinuous transfer buffer systems

The discontinuous buffer system is suitable for native PAGE, SDS-PAGE and IEF gels. The procedure is based on Kyhse-Andersen, 1984 (Kyhse-Andersen, J. (1984); J. Biochem. Biophys. Meth. 10, 203–209).

The low concentration of the SDS in the cathode buffer (0.01 %) does not cause denaturation of the proteins during the short contact phase.

Observe the following information when using discontinuous transfer buffer systems:
- Proteins over 80 kD: Before the blotting process, equilibrate in the cathode buffer for 5–10 minutes
- Urea IEF gels: Before the blotting process, equilibrate in cathode buffer to flush out urea

NOTICE! The proteins can diffuse out of the gel with too long equilibration times.

Anode buffer I	■ 300 mM tris/HCl, pH 10.4 ■ 20 % (v/v) methanol
Anode buffer II	■ 25 mM tris/HCl, pH = 10.4 ■ 20 % (v/v) methanol
Cathode buffer	■ 25 mM tris/HCl, pH = 9.4 ■ 40 mM caproic acid ■ 20 % (v/v) methanol ■ 0.01 % SDS
Alternative cathode buffer	■ 40 mM D, L-norleucin ■ (Dissolve norleucin while heating. Master solutions cannot be produced due to limited solubility!) ■ 25 mM tris/HCl, pH = 9.4 ■ 25 % (v/v) methanol

6.3.2 Assembling the blotting sandwich

CAUTION

Danger of skin injury and risk of poor blotting results

Skin contact with the chemical and the membrane can lead to skin injury.
Skin contact with the membrane can leave behind fingerprints and result in poor blotting results.

- Wear gloves when assembling the blotting sandwich and handling the membrane!

NOTICE

Avoid inclusion of air bubbles between membrane, blotting paper and gel. Air bubbles can lead to white spots on the membrane.

At the end of this work step, the blotting sandwich prepared for the blotting process should have the configuration shown in the following illustrations. The configuration depends on whether a continuous or discontinuous buffer system is used.

Blotting sandwich for a continuous buffer system

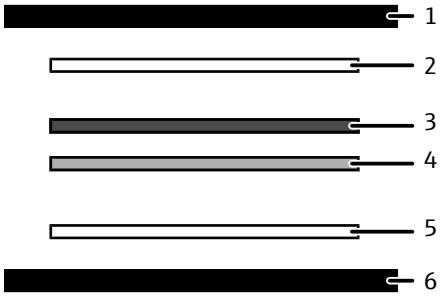

Fig. 4 Blotting sandwich for a continuous buffer system

1 Cathode in blotter cover
2 Filter paper
3 Gel
4 Membrane
5 Filter paper
6 Anode in device body

Blotting sandwich for a discontinuous buffer system

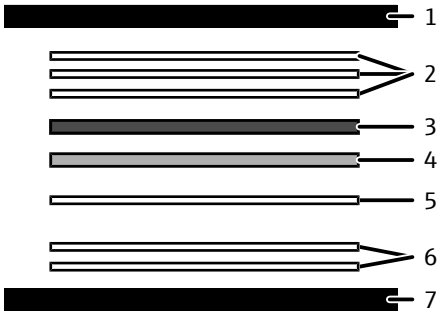

Fig. 5 Blotting sandwich for a discontinuous buffer system

1 Cathode in blotter cover
2 Filter paper with cathode buffer
3 Gel
4 Membrane
5 Filter paper with anode buffer II
6 Filter paper with anode buffer I
7 Anode in device body

Preparing the materials for the blotting sandwich

⚠ CAUTION! Wear gloves to assemble the blotting sandwich!

Proceed as follows to prepare the blotting sandwich:

- Separate the gel from any remaining glass plates.
- Cut out the part of the gel to blot.
- Cut a sufficient amount of blotting paper to the size of the gel.
  - Blotting paper with 0.34 mm thickness: 5 layers (e.g., Whatman 3MM Chr)
  - Blotting paper with 0.92 mm thickness: 3 layers (e.g., Whatman 17Chr)
  - Blotting paper with 1.20 mm thickness: 2 layers (e.g., Whatman GB005)
- Soak the blotting paper pieces in transfer buffer.

Assembling the blotting sandwich

⚠ CAUTION! Wear gloves to assemble the blotting sandwich!

Proceed as follows to assemble the blotting sandwich:

- Place the soaked pieces of **blotting paper** on the anode (+). The anode is the electrode on the device body.
- Carefully cut a **membrane** to the size of the gel. The standard membrane is nitrocellulose.

⚠ CAUTION! Only touch the membrane with gloves!

- Membrane made of PVDF: Before soaking in transfer buffer, incubate in methanol for 1 to 2 seconds. Then soak for 5 minutes in distilled water.
- Soak the membrane in transfer buffer for 5 minutes.
- Place the soaked membrane on the blotting paper pieces (anode side). Ensure that no air bubbles are caught between the membrane and the blotting paper.
- Equilibrate the **gel** for 5 minutes in transfer buffer. When using discontinuous buffer systems: Equilibrate the gel in cathode buffer.

ℹ NOTICE! Shorter or longer incubation times for the gel in the buffer affect the transfer efficiency. Keep the times for equilibration constant.

- Place the gel on the membrane. Ensure that no air bubbles are caught between the membrane and the gel.
- Cut another sufficient amount of **blotting paper** to the size of the gel.
  - Blotting paper with 0.34 mm thickness: 5 layers (e.g., Whatman 3MM Chr)
  - Blotting paper with 0.92 mm thickness: 3 layers (e.g., Whatman 17Chr)
  - Blotting paper with 1.20 mm thickness: 2 layers (e.g., Whatman GB005)
- Soak the blotting paper pieces in transfer buffer.
- Place the soaked pieces of blotting paper on the gel.
- Carefully remove any air bubbles. To remove, carefully roll a clean test tube or a pipette over the blotting sandwich.
  - ✓ The blotting sandwich is complete.
- Place the cover on the device body. When placing the cover, make sure it is positioned precisely parallel to the device body.
- When using thicker gels: Place a weight of 1 to 2 kg on the cover. A beaker of cold water can be used to weigh everything down.
  - ✓ The device is ready for the blotting process.

6.3.3 Connecting a cooling flow

The Biometra Fastblot B43 model has connections for continuous cooling.

ℹ NOTICE! Never confuse the inlet and outlet of the continuous cooling! The connections for the continuous cooling have different inner diameters: The inlet has a smaller diameter than the water outlet.

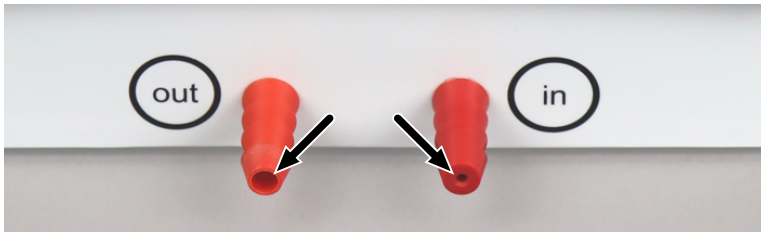

Fig. 6 Connections for continuous-flow cooling on the rear of the Fastblot B43

Continuous cooling requirements

Observe the following requirements:

Flow rate	0.5 to 1.0 l/min (without pressure)
Cooling agent	■ Water ■ **No** alcohol ■ **No** organic solvents

Recommended continuous cooling temperature

Additionally, the following temperature is recommended for best cooling:

Temperature	5 °C (with the use of a thermostat)

6.3.4 Starting and ending the blotting process

WARNING

Risk of electrical shock

High voltages are present in the interior of the device, which can lead to electric shock if contacted.

- Do not remove the cover from the blotter while the blotting process is running and the power supply device is connected!

NOTICE

Never leave the device active and unattended for too long (run time > 2 h). The heat generated during operation can damage the device.

Starting the blotting process

⇨ The device with blotting sandwich is prepared for the blotting process.

▸ Connect the power cables of the power supply device to the blotter.

NOTICE! Ensure correct electrode connection! Confusing the electrodes can result in device damage. The anode (+) is located on the device body, the cathode (-) is in the cover of the device.

▸ Switch on the power supply device and set it to ensure the following values:

- Constant current ≤ 5 mA/cm^2 gel surface
- Maximum power of 10 W

✓ The blotting process is started.

During the blotting process

If the cover heats up during the blotting process, reduce the current or place a pre-cooled cooling pad on it.

The maximum operating temperature must not exceed 50 °C!

Ending the blotting process

- Switch off the power supply device.
- Disconnect the power cables from the blotter.
- If continuous cooling is connected: Switch off continuous cooling.
- Remove the cover from the blotter.
- Carefully remove the blotting sandwich.
- Carefully remove the membrane from the layers of the blotting sandwich.
  - ✓ The membrane can be used for staining the immunoassays.

NOTICE! Clean the electrode plates after every blotting process with distilled water. Never use organic solvents! The electrodes can be dried with the aid of a paper towel.

6.4 Visualization

There are a number of methods for visualization of blotted sample in the technical literature, for example:

- Staining, for example with Coomassie Brilliant Blue
- Marking with radioactive probes
- Specific marking with hybridization probes

You can apply a visualization method to the membrane as soon as the blotting process is finished.

NOTICE! Observe that some visualization methods require preparation time. For example, the staining solution for Coomassie Brilliant Blue must be prepared at least one day before use, as it must be agitated overnight.

7 Maintenance and care

7.1 Cleaning

The device contains sensitive components. Observe the following information when cleaning the device to avoid damage:

- Never immerse the device in water!
- Never autoclave the device!
- Never use the device in a microwave!
- Clean the electrodes with a soft cloth after every transfer. The cloth can be dampened with distilled water.
- The anode plate is platinum-plated and very sensitive. Take particular care when cleaning the anode plate. Do not use abrasives or strong detergent.
- The cathode plate is made of special stainless steel. If necessary, you can clean the cathode plate with a light abrasive, for example ceramic stovetop cleaner.
- Salt deposits can build up on the device. Clean the complete device at regular intervals with slightly warm water to remove salt residue.
- Never use alcohol > 10 % (e.g., methanol, ethanol) or organic solvents (e.g., acetone, chloroform, toluol, benzol) to clean the housing or the electrodes. The device components are not resistant to these substances.

7.2 Maintenance

Regular maintenance of the blotter is not required.

If the electrodes become clogged after frequent use, making transfer inhomogeneous or decreasing it, contact Analytik Jena Service.

8 Returning the product

NOTICE

Risk of device damage due to unsuitable packaging material

- Only transport the device and its components in the original packaging.
- Empty the device completely and attach all transport locks before transporting the device.

- Clean all device components from biologically hazardous, chemical, and radioactive contamination.
- When registering the return, you will receive a decontamination declaration from customer service. Complete the declaration and attach the signed decontamination declaration to the outside of the shipment.
- Only use the original packaging for the shipment and insert the transport lock. If the original packaging is no longer available, please contact Analytik Jena or your local distributor.
- Attach the following warning label to the packaging:
  "**CAUTION! SENSITIVE ELECTRONIC DEVICE!**".
- Enclose a sheet with the following data:
  - Name and address of the sender
  - Name and telephone number of a contact for inquiries
  - A detailed description of the fault, the precise conditions and situations under which the fault occurs

9 Disposal

At the end of its service life, the device and its electronic components must be disposed of as electronic waste in accordance with the applicable regulations.

10 Specifications

10.1 Technical data

General device data

Housing material	PMMA
Anode material	Platinum-plated titanium
Cathode material	Special stainless steel
Dimensions ■ Fastblot B43 ■ Fastblot B44	 ■ 26 cm x 22 cm x 11 cm (LxWxH) ■ 24 cm x 22 cm x 11 cm (LxWxH)
Weight	approx. 2.5 kg
Maximum gel size	15.5 cm x19.5 cm

Operating conditions

Max. voltage	50 V
Max. current per cm^2 of gel	5 mA
Max. power	10 W For max. 30 min. operating time: 20 W Biometra Fastblot B43: For max. 30 min. and with cooling at 12 °C: 30 W
Max. temperature	50 °C
pH range (electrodes)	pH 3 to 10
Max. coolant flow rate	0.5 to 1.0 l/min (without pressure)
Max. transfer time	2 h

10.2 Ambient conditions

Work environment	Only designed for indoor use.
Ambient temperature	+ 5 °C to + 40 °C
Relative humidity	Max. 80 % (≤ 31 °C), with linear decrease to 50 (at 40 °C)
Maximum altitude	2000 m above sea level

10.3 Standards and directives

Protection type	The housing is protection type IP 20.
Device safety	The device complies with the following safety standard ■ EN 61010-1
Guidelines for China	The device contains substances subject to regulation (according to the directive GB/T 26572-2011). Analytik Jena guarantees that, if the device is used as intended, these substances will not leak within the next 25 years and therefore will not pose a threat to the environment or health within this time period.
EU directives	The device meets the requirements of the Directive 2011/65/EU. The device is designed and tested in accordance with standards meeting the requirements of EU Directive 2014/35/EU. The device leaves the factory in a perfect condition with regard to safety. To maintain this condition and to ensure safe operation, the user must strictly observe the safety and operating instructions contained in this operating manual. For accessories delivered with the device and system components from other manufacturers, the information provided in their respective operating manuals has priority.

11 Revision overview

version	Effective date	Changes
A	10/2023	First version

List of figures