iBlot system

FOR FAST WESTERN BLOTTING

USER MANUAL

CE

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Products contents

Type of products

This manual is supplied with the iD Blot Protein Transfer Device (L03010).

iD Blot Protein Transfer Device Contents

The contents of the iD Blot Protein Transfer Device are listed below:

Component	Quantity
iD Blot Device	1
iD Blot Graphite Electrode (Installed inside the device)	1
Sponge Cushion (Installed inside the device)	2
Regional specific power cord	1
Forceps	1
Shovel	1
Shallow Tray	2

Upon Receiving the Instrument

Examine the unit carefully for any damage incurred during transit. File any damage claims with the carrier. The warranty does not cover in-transit damage.

iD Blot Protein Transfer Pads

The following iD Blot Protein Transfer Pads are available from Eurogentec:

Product	Cat. No.
iD Blot Protein Transfer Pads (Basic, without membrane, 20-pak)	ID-BLPAB1-020
iD Blot Protein Transfer Pads (Nitrocellulose, 20-pak)	ID-BLPAN1-020
iD Blot Protein Transfer Pads (PVDF, 20-pak)	ID-BLPAP1-020

The iD Blot Protein Transfer Pads come with the following components:

Product	Basic	Nitrocellulose	PVDF
iD Blot Pad	20	20	20
iD Nitrocellulose membranes (20 sheets)	-	1	-
PVDF membranes (20 sheets)	-	-	1
iD Blot Equilibration Buffer (125 ml)	2	2	2
Gel Window (66 mm×56 mm)	1	1	1
Gel Window (76 mm×64 mm)	1	1	1
Gel Window (88 mm×78 mm)	1	1	1
Absorbent Filter Paper	1	1	1
Sponge Cushion	2	2	2

Components for iD Blot Pad are as follows:

Component	Quantity
1× iD Blot Cathode Pad	1
1× iD Blot Anode Pad	1

Store the iD Blot Pads at room temperature. For best results, use the iD Blot Pads before the expiration date printed on the package.

iD Blot Graphite Electrode

The iD Blot Graphite Electrode (ID-BLETD1-001) is available separately from Eurogentec:

Product	Cat. No.
iD Blot Graphite Electrode	ID-BLETD1-001

For best results, when a graphite electrode has been used for 100 times of protein transfer, replace it with a new one.

iD Blot Equilibration Buffer

The iD Blot Equilibration Buffer (ID-BLEQB1-125, 125 ml) is available separately from Eurogentec:

Product	Cat. No.
iD Blot Equilibration Buffer	ID-BLEQB1-125

DO NOT start a run without properly assembled Transfer Stacks in place!

Product Specifications

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

iD Blot Protein Transfer Device Specifications

Weight:	1.6 kg
Dimensions:	325 mm (l) ×195 mm (w) ×70 mm (h)
Electrical Parameters:	100-120 V, 220-240 V, 50/60 Hz, 3.2 A
Built-in Features:	Digital Display, Alarm, Light LED
Compatibility:	Suitable for fast electroblotting of proteins from mini polyacrylamide gels to PVDF or nitrocellulose membranes
Materials:	Acrylonitrile Butadiene Styrene, Polycarbonate, Aluminum, Titanium, Plasticized silicone.
Operating Temperature:	15 - 40 °C
Forceps:	Stainless steel
Shovel:	Polycarbonate
Shallow Tray:	Polycarbonate

Avoid acetone, dimethyl sulfoxide, and acetic acid. These reagents can erode or damage the device.

iD Blot Protein Transfer Pads Specifications

The iD Blot Protein Transfer Pads are used with the iD Blot Protein Transfer Device.

The specifications of the iD Blot Protein Transfer Pads are listed below:

iD Blot Protein Transfer Pad	
iD Blot Anode Pad:	90 mm (l) × 80 mm (w) × 2.5 mm (thickness)
iD Blot Cathode Pad:	90 mm (l) × 80 mm (w) × 2.5 mm (thickness)
Materials:	Blotting filter paper presoaked with proprietary anode or cathode buffer
Membrane	
Nitrocellulose:	90 mm (l) × 80 mm (w)
PVDF:	90 mm (l) × 80 mm (w)
iD Blot Equilibration Buffer	
Size:	125 ml × 2
Formulation	Proprietary

Gel Window	
Inter frame size:	66 mm × 56 mm
	76 mm × 64 mm
	88 mm × 78 mm
Materials:	Polycarbonate
Absorbent Filter Paper	
Size:	80 mm × 70 mm
Materials:	Vegetable fiber

iD Blot Graphite Electrode Specifications

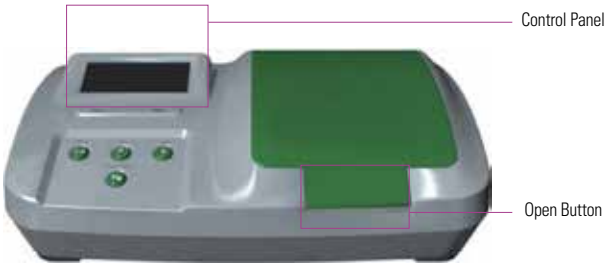
The iD Blot Electrode is used as the replaceable anode electrode of iD Blot Protein Transfer Device and available separately from Eurogentec.
The specifications for iD Blot Graphite Electrode are listed below:

iD Blot Graphite Electrode	
Dimensions:	105 mm (l) × 95 mm (w) × 10 mm (h)
Weight:	140 g
Materials:	Powdered carbon, Clay, and Stainless steel

iD Blot Protein Transfer Device

Front View of iD Blot Device

The front-top view showing various parts of the iD Blot Protein Transfer Device is shown below.



Rear View of the iD Blot Device

The rear view showing various parts of the iD Blot Protein Transfer Device is shown below.

Control Panel of the iD Blot Device

The Digital Display shows two rows of multi-digits that specify the transfer conditions as follows:

The upper three digits after text <PN> indicate how many times of transfer the graphite electrode has been used for.

The lower four digits specify the running time of protein transfer in minute and second.

The two Status Lights show the working mode of the iD Blot Protein Transfer Device. When the right status light is on, the device is switched on and working at transferring mode; when both left and right status lights are on, the device is working at numbering mode.

The **Reset** button is used to clear parameters. The **Min.** button is used to shift between transferring and numbering mode, and to set running time. Each short press will increase one minute. Each long press (2 seconds) will toggle working mode from transferring to numbering or the opposite. The **Sec.** button is also used to set running time, each press will increase 5 seconds.

The **Start/Stop** button is used to activate/stop the transfer program.

Top View of the Open iD Blot Device

The top view of open iD Blot Protein Transfer Device identifying various parts.

Accessory Products

ID Western H Detection Kits

The ID-Western H Detection Kits used for Western blotting analysis are available from Eurogentec. For more information, visit www.eurogentec.com or call the Technical Support.

Product	Cat#	Quantity
iD Western 1H - Essential Kit (Rabbit)	ID-WBESR1-005	1Kit (5 Assays)
iD Western 1H - Enhanced Kit with HRP (Rabbit)	ID-WBEHR1-005	1Kit (5 assays)
iD Western 1H - Enhanced Kit with TMB (Rabbit)	ID-WBETR1-005	1Kit (5 Assays)
iD Western 1H - Sensitive Kit with HRP (Rabbit)	ID-WBSHR1-005	1Kit (5 Assays)
iD Western 1H - Essential Kit (Mouse)	ID-WBESM1-005	1Kit (5 Assays)
iD Western 1H - Enhanced Kit with HRP (Mouse)	ID-WBEHM1-005	1Kit (5 assays)
iD Western 1H - Enhanced Kit with TMB (Mouse)	ID-WBETM1-005	1Kit (5 Assays)
iD Western 1H - Sensitive Kit with HRP (Mouse)	ID-WBSHM1-005	1Kit (5 Assays)
iD Western 1H - Essential Kit (Goat)	ID-WBESG1-005	1kit (5 assays)
iD Western 1H - Enhanced Kit with HRP (Goat)	ID-WBEHG1-005	1Kit (5 Assays)
iD Western 1H - Enhanced Kit with TMB (Goat)	ID-WBETG1-005	1Kit (5 assays)
iD Western 1H - Sensitive Kit with HRP (Goat)	ID-WBSHG1-005	1Kit (5 Assays)
iD Western 1H - Kit for Fluorescent Detection	ID-WBFLU1-010	1Kit (10 Assays)
iD Western 1H - Multiplex Kit for Fluorescent Detection	ID-WBMFL1-010	1Kit (10 Assays)

Introduction

System Overviews

Semi-dry Western blotting is a common technique applied in protein research. Conventional semi-dry blotting is a cumbersome process, requiring time -consuming reagent preparation and setup, followed by an electrophoretic transfer that could take one hour or more. Eurogentec's iD Blot Protein Transfer System accelerates the semi-dry blotting process without sacrificing performance. The iD Blot Protein Transfer System, consisting of the iD Blot Device and iD Blot Pads, enables researchers to quickly, reliably perform electrophoretic transfer of proteins from various types of mini polyacrylamide gels to membranes in 9 to 12 minutes without the need to prepare additional buffers.The proteins transferred using the iD Blot Protein Transfer System exhibit high detection sensitivity as to proteins transferred using other existing blotting methods.

System Components

The iD Blot Protein Transfer System consists of:

- iD Blot Device

The iD Blot Device is a self-contained electroblotting unit with a power supply built-in for fast transfer of proteins from poly-acrylamide gels to membranes (PVDF or nitrocellulose).

- iD Blot Pads

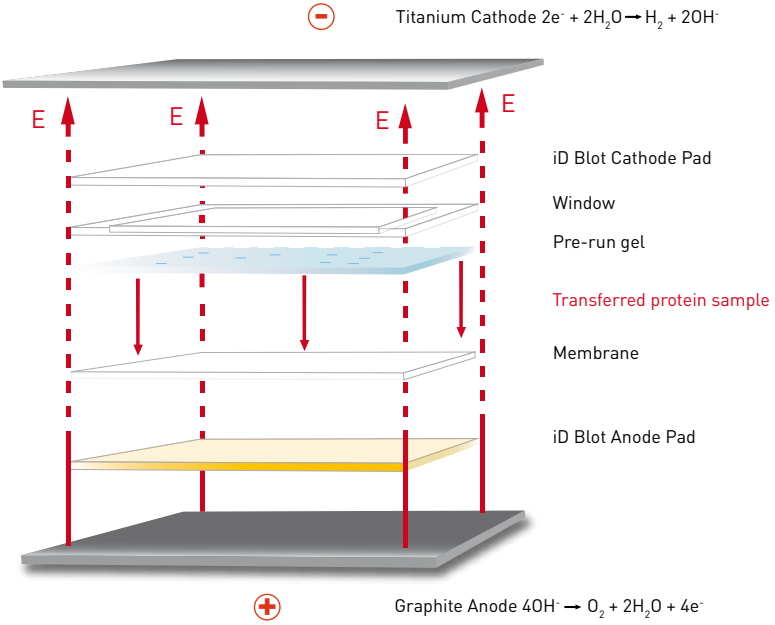
As the consumable part of iD Blot Protein Transfer System, the iD Blot Protein Transfer Pads are the mixed assortment of iD Blot Protein Transfer Pad, nitrocellulose or PVDF membranes, Equilibration Buffer and Gel Window. Each pack of iD Blot Pad contains an 1×iD Blot Cathode Pad and an 1×iD Blot Anode Pad presoaked with proprietary cathode buffer and anode buffer respectively, allowing for rapid, convenient and reliable protein blotting without the need to prepare additional buffers.

System Mechanism

To use the iD Blot Protein Transfer System for rapid protein blotting, assemble the iD Blot Protein Transfer Pad with your pre-run gel and the membrane on the iD Blot Protein Transfer Device. The iD Blot Cathode Pad and iD Blot Anode Pad act as ion reservoirs that contain the appropriate anode and cathode buffers. The design of the iD Blot Device reduces the distance between electrodes and the integrated power supply enables the system to generate a certain definite voltage allowing for rapid and directional movement of negatively charged protein molecules from the gel matrix onto

the membrane within 9-12 minutes. Meanwhile, the membrane presoaked with the iD Blot Equilibration Buffer shows good combination ability with proteins that prevents the flow through of low molecular weight proteins from the membrane.

Schematic mechanism of the iD Blot Protein Transfer System showing the flow of current:



System Features

- Unique semi-dry electroblotting technique created for fast, reliable protein transfer within 9-12 minutes.
- Self-contained electroblotting unit with an integrated power supply for easy and convenient procedures.
- Consumable transfer pads offering convenience without the need for additional buffers.
- Compatible for use with various types of mini polyacrylamide gels.
- Proprietary formula without methanol.
- High transfer efficiency as compared to other existing blotting methods.

Quick Reference Guide

Mode	Action	Sound	Light	Display
iD Blot Device plugged in	Connect the iD Blot Device to an electrical outlet and power switch is on	-	Steady right light	Default running time (00:00)
iD Blot Device and transfer stack assembled	Assemble transfer stack on the device and close lid	-	Steady right light	Default running time (00:00)
Time selection	Press Min. and Sec. button to select desired running time	-	Steady right light	User specified running time (00:00)
Run	Press Start / Stop button	Continuous beeping for 2 minutes	Flashing right light	Counting down time
End of run	Automatic	Continuous beeping for 2 minutes	Steady right light	Default running time
Checking the number of uses of the graphite anode	Press and hold Min. button for 2 seconds	-	Steady left and right lights	Times of the graphite anode has been used for transfer
Replacement of worn graphite anode	Switch off the device and replace the worn graphite with a new one	-	-	-

Protocols

Recommendations

To obtain the best results, follow these recommendations:

▷ 1.Wear gloves at all times during the entire blotting procedures to prevent contamination of pads, gels and membranes.

▷ 2.Do not touch the gel or membrane with bare or gloved hands. This may contaminate the gel or membrane and interfere with further analysis. If needed, always use forceps to adjust the membrane or gel.

▷ 3.Avoid using expired iD Blot Pads and iD Blot Equilibration Buffer. Always use the pads and buffer before the specified expiration date printed on the package.

▷ 4.Remove any trapped air bubbles between the gel and membrane during the assembly of the transfer stack using the small shovel supplied with the device.

Installing the ID Blot Device

▷ 1.Check the Power Cord supplied with the unit to ensure that the cord is compatible with the local socket format.

▷ 2.Place the iD Blot Protein Transfer Device on a levelled laboratory bench. Keep the area around the device clear to ensure proper ventilation of the unit.

▷ 3.For your safety: Position the device properly such that the Power Switch and the AC inlet located on the rear of the unit are easily accessible.

▷ 4.Ensure the Power Switch is in the Off position.

▷ 5.Open the closed lid of the iD Blot Protein Transfer Device by pressing the Open button. Place one or two pieces of Sponge Cushion in the anode tank depending on gel thickness. For 1.5 mm gel, use one piece of Sponge Cushion; for 0.75 and 1.0 mm gel, use two pieces of Sponge Cushion. ▷ 5

▷ 6.Insert the iD Blot Graphite Electrode into the anode tank as described in Section "Replacing the iD Blot Graphite Electrode", then close the lid of the device.

▷ 7.Pull out the Waste Tray from the right side of the device. Place a new Absorbent Filter Paper inside the tray and then push the tray in. ▷ 7

▷ 5 Note: After 20 times of protein transfer, replace the used Sponge Cushions with new ones. A pair of new Sponge Cushions are included in each box of iD Blot Protein Transfer Pads.

▷ 7 Note: Change absorbent paper with a new one when opening a new box of pads.

▷ 8.Attach the power cord to the AC inlet and then to the electrical outlet. Use only properly grounded AC outlets and power cords.

▷ 9.When the electrophoresis of your samples is almost complete, press the Power Switch (located on the rear of the device) to turn ON the iD Blot Device. The right Status Light is on indicating you are using transfer mode. The lower four digits of the Digital Display show the default running time (00:00).

You are ready to use the iD Blot Device for blotting application.

Assembling the Transfer Stack

▷ 1. Open the closed lid by pressing the Open button.

▷ 2.Remove one package labeled as iD Blot Pad from the iD Blot Transfer Pads box and tear the laminated sealing of the package. Remove the two small packages respectively labeled 1×iD Blot Cathode Pad and 1×iD Blot Anode Pad.

▷ 3. Tear the sealing of the 1×iD Blot Anode Pad package. Remove the iD Blot Anode Pad from the package and place it on the anode plate of the iD Blot Protein Transfer Device.

▷ 4.Pour 10 ml iD Blot Equilibration Buffer into the shallow tray supplied with the iD Blot Device.

▷ 5.Tear the sealing of the Nitrocellulose or PVDF Membranes package. Remove one sheet of membrane and soak it in the iD Blot Equilibration Buffer for 1 minute. ▷ 5

▷ 6.Place the equilibrated membrane on the iD Blot Anode Pad. Gently remove air bubbles between the membrane and the anode pad using the small shovel supplied with the device.

▷ 5 Note: If a PVDF membrane is used, the membrane must be pre-wetted with methanol before equilibrating in iD Blot Equilibration Buffer. For users of iD Blot Pads (Basic, 20-pak) (ID-BLPAB1-020), the precut membrane should be prepared by themselves.

▷ 7.Carefully remove the pre-run gel containing your protein samples from the gel cassette and briefly rinse the gel with distilled water in another shallow tray to remove any small gel pieces attached to the gel and facilitate easy positioning of the gel on the membrane

▷ 8.Place the gel on the membrane. Gently remove air bubbles between the gel and the membrane.

▷ 9. Select appropriate Gel Window according to actual size of the gel (see the table below). Place Gel Window on the gel. Ensure that the Gel Window fully covers the margin of the membrane. ▷ 9

▷ 9 Note: Gel Window is used as the spacer between anode pad and cathode pad to prevent short circuit.

Pre-run gel	Gel Window
Gel with size > 80 × 60 mm	Gel Window 88 × 78 mm
Gel with size 66 × 56 mm to 80 × 60 mm	Gel Window 76 × 64 mm
Gel with size < 66 × 56 mm	Gel Window 66 × 56 mm

▷ 10 Note: During assembling of the transfer stack, make sure to remove all the air bubbles trapped between the transfer pads, pre-run gel and the membrane, which may prevent the transfer of proteins and cause empty spots on the transferred membrane.

▷ 10. Tear the sealing of the 1×iD Blot Cathode Pad package. Remove the iD Blot Cathode Pad from the package and place it on the gel.

▷ 11. Press the Open button, and then push back and close the lid of the iD Blot Device.

Performing Blotting

Perform protein blotting as described below within 15 minutes of assembling the transfer stack.

▷ 1 Note: Based on the initial results, the transfer time may need to be optimized to make best transfer results by pressing the Time button in 5-second increment. For proteins greater than 200 kDa, we recommend to start with 11 minutes.

▷ 1.Press the Min. and Sec. buttons to set appropriate running time based on the protein size (see table below). If an undesired running time is set by mistake, press Reset button to clear the wrong time, and then press the Min. and Sec. buttons to choose the desired running time. ▷ 1

Protein size (kDa)	Recommended start running time (min)
< 80	7
80-160mm	8-9
> 160	10

▷ 2. Press the Start/Stop button to start the transfer. The running time begins to count down and right Status Light keeps flashing during the whole transfer program.

▷ 3. At the end of the transfer, current automatically shuts off and the iD Blot Protein Transfer Device signals the end of transfer with repeated beeping sound. The right status light stops flashing and the lower five digits show text (00:00).
▷ 4. Press any button on the control panel to stop the beeping.
▷ 5. Proceed to disassemble the stack and clean the device.

Disassembling and Cleaning the iD Blot Device

To obtain good transfer and detection results, disassemble the transfer stack right away after ending the blotting procedure.

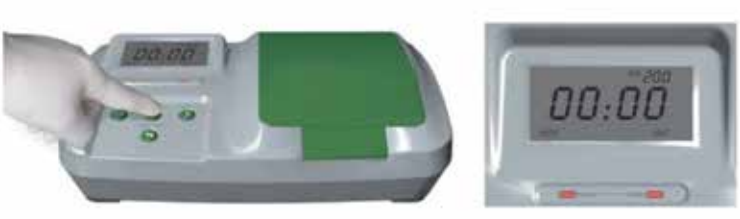
▷ 1. Open the closed lid by pressing the Open button.
▷ 2. Carefully separate the transferred membrane from the transfer stack and proceed with further protein detection procedures.▷ 2
▷ 3.Discard the gel and the used iD Blot Protein Transfer Pad. ▷ 3
▷ 4.Clean the titanium cathode plate, graphite anode plate and its surrounding area with a dry cloth or paper tissue.
▷ 5.Replace the Absorbent Filter Paper in the Waste Tray with a new one when it has soaked up the waste from 20 times of transfer. Pull out the WasteTray from the right side of the device to perform the replacement. ▷ 5
At this point, the iD Blot Device is ready for another run.
If you are not using the device, turn off the Power Switch located on the back of the device.
For any other repairs and service, contact Technical Support. Do not perform any repairs or service on the iD Blot Device by yourself to avoid any possible damages to the device.

▷ 2 Note: If you are using PVDF membrane, place the membrane immediately into the blocking solution (or water) as PVDF membrane dries quickly. If the PVDF membrane is dried, rewet the membrane with methanol and then rinse it with distilled water to wet it completely before use.
▷ 3 Note: Do not re-use the iD Blot Pad after blotting. Discard after each use.
▷ 5 Note: After about 40 runs, take out the graphite electrode, soak in distilled water for 30 minutes, dry the surface with a clean paper towel, then leave air dry overnight with graphite side up.

Replacing the Graphite Electrode

During the transfer process, the Graphite Electrode will absorb ions from anode pad as well as lose carbon composition of the anode buffer. For best blotting results, after having been used for 100 times of transfer, the worn Graphite Electrode should be replaced by a new one.

▷ 1. If the iD Blot Protein Transfer Device works at transferring mode, press and hold Min. button for 2 seconds to toggle to numbering mode. If the upper three digits show "100" or a number greater than "100", perform the replacing protocol as describe below.



▷ 2.Switch Off the iD Blot Protein Transfer Device. Open the lid of the device and take the worn Graphite Electrode out of the device.

▷ 3. Tear the sealing of a new Graphite Electrode package and take the new Graphite Electrode out of the package. Place the new Graphite Electrode into the anode tank and close the lid of the device.

▷ 4. Switch On the iD Blot Device. Press and hold Min. button for 2 seconds to toggle to numbering mode. When the upper three digits are flashing, press Reset button to zero the transferring times.

After successfully installing Graphite Electrode into the iD Blot Device, you are ready to use the iD Blot Device for another blotting application.

Troubleshooting

Problem	Cause	Solution
The right Status Light doesn't flash during blotting process.	Incomplete electric circuit due to improper assembly of the transfer pads.	Ensure the transfer stack is assembled correctly: use the iD Blot Anode Pad first followed by the membrane, the pre-run gel, Gel Window and iD Blot Cathode Pad.
The left and right Status Lights flash simultaneously	Excessive current is flowing through the Device.	Check the transfer stack and ensure Gel Window covered correctly on the gel.
Inefficient transfer	1.Salt built-up on plate electrodes	1.Clean the titanium cathode plate, graphite anode plate with a wet cloth or paper tissue followed by a dry one to remove any insoluble salts.
	2.Membrane insufficiently equilibrated in iD Blot Equilibration buffer	2.Equilibrate membrane in iD Blot Equilibration buffer before transfer
	3.Incorrect transfer conditions or insufficient transfer time	3.Use a gel of lower concentration to separate high molecular weight proteins. Increase the transfer time in 5-second increments.
	4. PVDF membrane was not prewet with methanol	4.Prewet PVDF membrane with methonal before transfer.
	5.Confusion of the iD Blot Anode Pad and Cathode Pad	5.Ensure the transfer stack is assembled correctly: Bottom-iD Blot Anode Pad (yellow), Top-iD Blot Cathode Pad (white).
Empty spots on the membrane	Air bubbles trapped between gel and membrane prevent the transfer of proteins.	When assembling transfer stack, use the small shovel supplied with the device to remove any air bubbles between the gel and the membrane.

iD Blot Protein Transfer Device

Eurogentec warrants that iD Blot Protein Transfer Device will be free from defects in material and workmanship for a period of one year from date of purchase. If any defects occur in the product during this warranty period, Eurogentec will, at its option, repair, replace, or refund the purchase price of this product at no charge to you. The following defects, however, are specifically excluded:

- 1.Defects caused by improper operation.
- 2.Repair or modification done by anyone other than Eurogentec or an authorized agent.
- 3.Use of fittings or other spare parts supplied by anyone other than Eurogentec.
- 4.Damage caused by accident or misuse.
- 5.Damage caused by disaster.
- 6.Corrosion due to use of improper solvent or sample.

For any inquiry or request for repair service, contact Eurogentec after confirming the model and serial number of your instrument. For your protection, items being returned must be insured against possible damage or loss. This warranty shall be limited to the replacement of defective products. It is expressly agreed that this warranty will be in lieu of all warranties of fitness and in lieu of the warranty of merchantability.

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FOR FAST WESTERN BLOTTING

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