Title: The Operation of the iBlot™ Dry Blotting System

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Table of Contents

1.0 Purpose

This document describes the operation, and maintenance of the iBlot™ Dry Blotting System.

2.0 Scope

This procedure covers blotting of mini gels or 1 midi gels of 1.0 and 1.5 mm thickness subsequent to the assembly of the iBlot™ Gel Stacks using a Blotting Roller. This procedure does not include instructions for use of the Debubbling Roller of the iBlot™ Gel Transfer Device.

3.0 Authority and Responsibility

3.1 The Director, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.

3.2 PA/QC is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BOA).

3.3 PA/QC personnel are responsible for the performance of this procedure.

3.4 PA/QC is responsible for reviewing the data and documentation of the results of this

procedure.

3.5 BQA is responsible for quality oversight of this operation.

4.0 Materials and Equipment

4.1 IBlot™ Gel Transfer Device

4.2 IBlot™ Ge Transfer Stacks consist of iBlot™ Cathode Stack (Top), iBlot™ Disposable Sponge, iBlot™ Filter Paper, and iBlot™ Anode Stack (Bottom). Available blotting membrane materials and sizes are the following:

4.2.1 PVDF Membrane Stacks, Regular, BDP PN 30843 for blotting two (2) mini gels simultaneously, or one midi gel.

4.2.2 PVDF Membrane Stacks, Mini, BDP PN 30855 for blotting one (1) mini gel.

4.2.3 Nitrocellulose Membrane Stack, Regular, BDP PN 30844, for blotting two (2) mini gels simultaneously, or one midi gel.

4.2.4 Nitrocellulose Membrane Stack, Mini, PN 30856 for blotting one (1) mini gel.

4.3 Blotting Roller supplied with the Gel Transfer Device.

4.4 Electrophoresed gels (completed within 72 hours prior to blotting) of any of the following compatible sizes and types containing protein samples, and standards, as according to the product specific SOP and MS-AP requirements:

4.4.1 Mini NuPAGE® Bis-Tris or Tris-Acetate, Tricine, or Tris-Glycine.

4.4.2 Midi NuPAGE® Novex® Bis-Tris or Tris-Acetate, or Tris-Glycine.

4.5 Gel Knife.

4.6 Forceps.

4.7 Reagent Grade or BDP approved de-ionized water.

4.8 NuPAGE® Transfer Buffer, BDP PN 30079, or BDP approved equivalent.

4.9 Methanol, BDP PN 10115 or BDP approved equivalent.

4.10 NuPAGE® Antioxidant, BDP PN 30076 or BDP approved equivalent.

4.11 Clean containers.

5.0 Procedure

5.1 Open the lid of the iBlot™ gel transfer device. Ensure that the blotting surface is clean and dry. If necessary, clean the surface with a damp towel/utility wipe and allow it to dry before use.

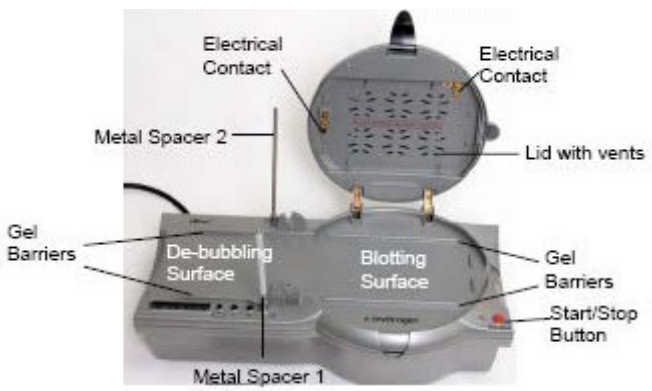

Figure 1: IBlot™ Gel Transfer Device

5.2 Turn on the power switch of the iBlot™ transfer device prior to completion of electrophoresis of the samples. Ensure that the fan of the device is running and the digital displays the default parameters used with the last program run.

5.3 Select a gel transfer stack of appropriate material, and size according to section 4.2.

5.4 Prior to assembling the gel transfer stacks and gel(s), select the appropriate parameters based on gel type, or the pre-determined optimized blotting conditions on the iBlot™ device. The iBlot™ Gel Transfer Device is pre-programmed with the following 5 blotting programs which allow the use of different combinations of voltage and time.

Program	Volts	Default Run Time (Minutes)	Run Time Limit (Minutes)
P1	25	6	10
P2	23	6	11
P3	20	7	13
P4	15	7	16
P5	10	7	25

Table 1: Installed Programs of the iBlot™ Device

5.5 Press the Select button to toggle between Program, Minutes and Seconds

Fig. 2 iBlot™ Device Control Panel

5.5.1 Once the selected item blinks, use the Up/Down (+ or -) Buttons to increase or decrease the values to the desired parameters. Note – Program parameters may not be changed while a blot program is running.

5.6 The following are recommended parameters for blotting mini gels containing protein of a range of sizes:

Size (kDa)	Program	Duration (Min)
≤ 80	P3	6 - 8
80 - 150	P3	7 - 9
≥ 150	P3	8 -10*

* equilibration may be necessary

5.6.1 Based on the specific properties of a protein sample, transfer of high molecular weight proteins > 150 KDa may require enhancement of the blotting process by equilibration of NuPAGE® or Tris-Glycine gels at room temperature for 10 - 20 minutes in 100 mL Equilibration Buffer consisting of 2x NuPAGE® Transfer Buffer containing 10% Methanol and 1:1000 NuPAGE® Antioxidant prior to the transfer process.

5.6.2 Whenever equilibration is needed, prepare the Equilibration Buffer per ***SOP 22702 – Solutions Used in Process Analytics*** as follows for 100 mL:

5.6.2.1 10 mL NuPAGE® Transfer Buffer (20x)

5.6.2.2 80 mL Deionized H2O

5.6.2.3 10 mL Methanol

5.6.2.4 100 µL NuPAGE® Antioxidant

5.6.3 Perform the equilibration of the gel in a clean container.

5.7 Remove the laminated sealing and place the Anode Stack (Bottom) with its plastic tray directly on the blotting surface, aligned to the gel barriers on the right.

Fig. 3 Removing laminated sealing from Anode (bottom) stack

5.8 Remove the pre-run gel from its cassette and place it on the transfer membrane of the anode stack with the lower end of the gel against the right border of the membrane.

5.8.1 If two mini gels are being blotted, place them on the transfer membranes head-to-head.

Fig 4. Two mini gels head-to-head

5.9 In a clean container, soak one iBlot™ filter paper in deionized water.

5.9.1 Place the pre-soaked filter paper on the pre-run gel(s) and use the blotting roller to remove any air bubbles trapped between the membrane and gel(s). Use extremely light pressure on the roller to remove air bubbles.

NOTE: When removing the air bubbles, do not apply excessive pressure and avoid repeated rolling on one area or increased background on the blot may result. See Figure 5.

Fig 5. Removal of Air Bubbles

5.10 Remove the iBlot™ Cathode Stack, (top) from the package. Discard the plastic tray. Place the Cathode Stack on top of the pre-soaked filter paper with the copper electrode side facing up. Ensure that the top stack is properly aligned to the right of the bottom stack.

NOTE: Always discard the plastic tray of the Cathode (top) Stack.

5.11 Remove any trapped air bubbles using the blotting roller.

NOTE: When removing the air bubbles, do not apply excessive pressure and avoid repeated rolling on one area or increased background on the blot may result. See Figure 6.

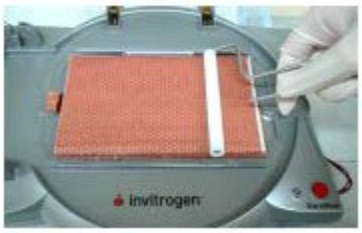

Fig. 6 Preparation of the Cathode (Top) Stack

5.12 Place the iBlot™ disposable sponge on the inner side of the lid, between the small protrusions, such that the metal contact is toward the upper right of the open lid.

5.13 Close the lid, and secure the latch of the iBlot™ Gel Transfer Device. The red light will indicate a closed circuit. Initiate the blotting process within 15 minutes of assembling the stacks with the gel.

5.13.1 Confirm that the correct program and duration have been selected in the display window.

5.13.2 Press the Start/Stop button to begin the transfer. The red light changes to green.

5.13.3 At the end of the transfer, the current shuts off and this green light changes into a flashing red light.

NOTE: To obtain good transfer and detection results, disassemble the device and stacks within 30 minutes of conclusion of the blotting procedure.

NOTE: Always don a fresh pair of gloves before disassembling the iBlot Gel Transfer Stack to prevent carry-over contamination of the blot.

5.14 Open the lid of the Blot™ Gel Transfer Device and discard the iBlot™ disposable sponge and Cathode Stack (Top).

5.15 Carefully remove and discard the filter paper and gel. Avoid touching the transfer membrane.

5.15.1 If two (2) mini gels are being transferred on a Regular stack, prior to removing the gels, use a sterile scalpel and gel knife to incise the membrane along the line convergence of the two gels to allow for separate analysis of the respective segments of blotted membranes.

5.15.2 Discrete immunodetection procedures may be performed on each half of the transfer blot.

5.16 Use a pair of forceps to remove the transfer membrane from the Anode Stack and proceed with the product specific detection procedure.

5.17 Discard the remaining parts of the Anode Stack (Bottom).

5.18 The Gel Transfer Device may be immediately used for blotting other gels if needed. No components of the gel stacks are reusable.

6.0 Maintenance

6.1 After all iBlot™ gel transfer runs have been concluded, turn off the power switch.

6.2 Clean the blotting surface with a damp cloth or paper and allow it to dry.

7.0 Documentation

7.1 Document the use of the iBlot™ Gel Transfer Device in its appropriate equipment log per ***SOP 21531 - Equipment Logs***.

7.2 Record reagent part and lot numbers used during the electro-blotting procedure on the product specific form(s) or laboratory notebook as applicable.

7.3 Record the iBlot instrument ID and program settings on the product specific form(s) or laboratory notebook as applicable.

8.0 References and Related Documents

8.1 **SOP 21409** *Good Documentation Practices*

8.2 **SOP 21531** *Equipment Logs*

8.3 **SOP 22702** *Solutions Used in Process Analytics*

8.4 http://tools.invitrogen.com/content/sfs/manuals/iblotsystem_man.pdf