

# Cat # NB-12-8101-MIDI4-NeoBlot Auto, Automated western blot processor, with 4 midi trays and touchscreen

#### **BlotCycler Instructions.**

Before you start, you need to run electrophoresis, transfer protein to membrane, and prepare the following solutions: mini trays midi trays delta trays Blocking solution for each blot 20-28 ml 8-15 ml 12-18 ml 18-25 ml Primary Antibody (PA) for each blot 8-16 ml 3-10 ml Secondary Antibody (SA) for each blot 12-18 ml 20-28 ml 5-15 ml **Washing buffer** 1.0-3.5 L (depends on the number of blots)

### A. Loading of the BlotCycler.

**Note**: If you are not going to use BlotCycler for several days run cleaning cycle first and then open the valves and turn the instrument off (see Section D).

*Important:* Before adding the solutions turn the BlotCycler on. If the machine was on, open the valves, turn it off, wait 10 sec, and turn it back on.

- Remove tray covers, place membranes in the trays, add blocking buffers, and close the trays with covers.
- Remove top cover.
- Add Primary antibodies (P1- P6, marked with letter P). Place collection vial for primary antibody.

Note: Make sure that PA and trays are matched.

Add Secondary Antibodies (S1 – S6, marked with letter S).

Note: Make sure that SA and trays are matched.

• Fill the tank with washer up to appropriate level, close the top cover.

# **B.** Protocol setup

Turn system OFF and ON to reset before adding any solutions or programming the instrument (see A).

Press "Run" to use current programs or "Protocol" to select custom program (see the full version of the manual for more details)

- Press "Select Custom Protocol"
- On the next screen make changes to protocol if needed and select side by pressing "Left" or "Right" buttons (on the bottom of the screen).

Note: Minimal blocking time 5 min, minimal antibody incubation time is 10 min, washing time could be set between 3 min and 20 min, the optimal washing time is 5 min.

• Return to home screen by pressing



#### C.Start the cycle.

Press "Run" and select the side by pressing "Start".

The status screen will indicate the current step in protocol by blinking red circle.

Note: Wait at least 10 sec after starting one side before starting an incubation protocol on the other side.

Note: To load the other side you can stop the motor for 2 min by pressing "stop shaking" button.

# D. Cleaning.

Note: you can do the cleaning only after all cycling is finished, one side at a time.

- Remove all membranes and collection vials.
- Fill the device with a diluted cleaning solution, CL500, make sure there is enough cleaning solution. Otherwise, the pumps may be destroyed.
- Press 'Maintenance" button on the home screen.
- On a new screen press "Cleaning" and select the side.

Note: Open the valves and restart BlotCycler before running a new protocol after cleaning.

Note: In order to drain buffers and the cleaning solution from the tank, remove a plug between the pumps.

Warning: in case of liquid spill immediately switch offthe system, carefully wipe out all spilled solution and do not operate until completely dry

# **Critical points:**

- 1. Run cleaning after each use.
- 2. Open all valves when cleaning is finished.
- 3. Restart the unit before each use to reset valves.
- 4. Make sure drain tubing do not touch solution.
- 5. If protocol is stopped during execution, it will restart from the beginning.

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