TECHNICAL NOTE

Automating ScanLater Western Blot Detection with the StakMax Microplate Handling System

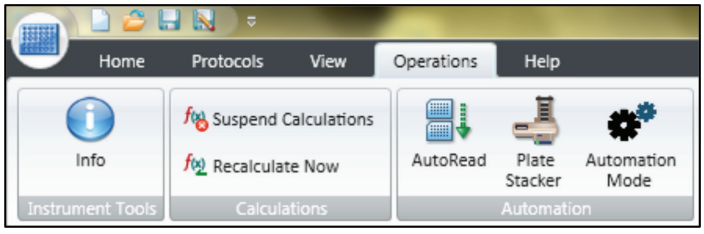
Introduction

The ScanLater Western Blot System improves upon the traditional western blot protocol by employing a europium-labeled secondary antibody that is detected by time-resolved fluorescence (TRF). This substrate-free detection method enables more accurate quantitation than enzymatic methods, as well as sensitivity for protein detection in the high femtogram range, which is comparable to traditional chemiluminescent western blot methods. Signal is highly stable, and the blot can be read up to 30 days after the final wash step. The ScanLater Western Blot Detection Cartridge can be added to any SpectraMax Paradigm, i3, or i3x Multi-Mode Microplate Reader.

The signal stability of the ScanLater system allows for high-throughput batch processing of western blots that is not possible with unstable, enzymatic detection methods. By pairing a ScanLater-equipped SpectraMax i3x reader with a StakMax Microplate Handling System, a stack of 20, 40, or even 50 blots can be read automatically. For this technical note, ScanLater blot holders loaded with western blot membranes were loaded into a 20-plate magazine. A custom script was written to handle data file formatting, plate handling, data generation, and data storage.

Script setup

In the Operations tab of SoftMax Pro Software, 'Plate Stacker' is selected.



In the StakMax window that opens, select 'Scripting' and choose file path 'New' to create a new script. This opens up a new window in the software with a menu of commands that can be combined to make a custom script. For ScanLater automation, the script should be set up as follows:

Command Script:

```
Open Protocol:C:\Users\Marketing\Desktop\Western blot auto.spr
Save Document As:Western blot test data.sda
Select Section:Plate1
Open Drawer
Put Plate
Close Drawer
Start Read
Return Plate
Begin Repeat:2
New Plate Section
Open Drawer
Put Plate
Close Drawer
Start Read
Return Plate
End Repeat
Save Document As:C:\Users\Western Lab\Desktop\ScanLater Data.sda
Restack
```

Before the script is run, a western blot protocol must be created. In the script above, the protocol is called 'Western blot auto.spr', and the first line of the script indicates its location so that it can be opened automatically by the software. The 'Save Document As' step is critical, as this creates a data (.sda) file along with a folder into which image (.tif) files for each blot are deposited. This step is required for automation to proceed.

Once the blots are read, the final data set is saved, and the plates are restacked to their original order. Data can then be analyzed through SoftMax Pro Software using a custom Excel analysis module for quantitative analysis.

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