SOP: SP071

Operation of the Typhoon Imager

Materials and Reagents:

1. Fluorescent Western Blot (SOP SP070)
2. Typhoon TRIO Imager

Protocol:

1. _____ Run Fluorescent western blot according to SOP SP070 developed with either Cy2 or Cy5 conjugated secondary antibody. Make sure to protect the blot from light at all times once the secondary antibody has been added. Failure to do so will result in a weakened signal.

2. _____ When final wash step is complete, the PVDF membrane can be scanner either while wet or after drying on paper towels. If dry, the membrane will be much more fragile.

3. _____ To detect the secondary antibody signal, use the Typhoon Imager located in room C319.

4. _____ If the imager is turned off, turn on before logging in and initiating software.

5. _____ Open scanner bed and clean with ethanol. Place blot faced down on scanner with the ladder on the right side.

6. _____ To use, login to the computer and click on the icon labeled "Typhoon Scanner Control v5.0"

7. _____ Change the acquisition mode to Fluorescence.

8. _____ In "setup" choose either:

   A: 670 BP 30 Cy5 for anti-rabbit secondary
   B: 520 BP 40 Cy2, ECL+ for anti-mouse secondary

9. _____ Change PMT to 400 (this can be raised if the signal is weak)
10. _____ Sensitivity can be left at normal, however this can also be altered to accommodate a weak signal
11. _____ Options: select orientation of the blot to be scanned. Typically this is the backwards R. (see step 5)
12. _____ Select area size to be scanned – 1-22 horizontal and A-R vertical.
13. _____ Hit scan button.
14. _____ After scan is complete, save file.
15. _____ The image can be manipulated or converted to a tiff file by opening the "Image Quant TL V2005".
    A: Select "1D gel analysis"
    B: Open file
    C: Edit image
    D: save as tiff