Western blot quantification by Image J

This is a simple protocol to quantitatively analyze western blot.

Procedure

1. Save original western blot image in JPEG, PNG or TIFF on your computer.

2. Open the Image J in the Application folder on your computer. If you do not have this software, you may down load from NIH website at: https://imagej.nih.gov/ij/download.html. Make sure you download the one that fits your computer.

3. Open the image from ImageJ.

4. Select the rectangle icon and define your quantification area for the biggest band.

5. Use this to quantify all bands and background nearby for each band using "Command M". The IntDen will show with the Area.

6. Copy and paste the numbers to Excel sheet and label the lanes with ID and treatment. Subtract the background value for each band to get Corrected IntDen.

7. Copy paste the image as a record.

8. Do step 1-6 for the loading control (Gapdh or alpha tubulin).

9. Divide the Corrected IntDen for your protein of interest by Corrected IntDen for the loading control to obtain Normalized IntDen.

10. Calculate the mean value of your control group in your gel image.

11. Divide all the values by this number to obtain fold change.

12. You can use = function below this part to duplicate these numbers and rearrange the data to put samples of the same group together to calculate Mean SE and perform T-test or other statistical analyses.

Note

1. For phosphoproteins, do the same calculations using total protein as your loading control, i.e. obtain fold change using total protein, such as total Akt for p-Akt(S473).

2. If you have multiple images with common control, you can divide your fold change by fold change of the common control to make the numbers comparable between images. You will then need to calculate the mean value of all the control samples and get fold change again for all the other groups.