DEEP PURPLE™ TOTAL PROTEIN STAIN

Solutuions needed:

Fix Solution

10% MeOH, 7.5 % Acetic Acid

For four liters:

400 ml MeOH
300 ml Acetic Acid
3300 ml mQWater

Wash Solution (for backed gels)

35 mM sodium bicarbonate, 300 mM sodium carbonate (should be pH 11)

For four liters:

11.76 gms sodium bicarbonate
192 gms sodium carbonate
dissolve in 4 liters mQWater
check pH

Wash Solution (for non-backed gels)

200 mM sodium carbonate

For one liter:

64 gms sodium carbonate
dissolve in 500 ml mQWater

Storage Solution

7.5% Acetic Acid

For four liters:

300 ml Acetic Acid
3700 ml mQWater

Procedure for Backed gels (large gels):

1. Incubate backed gel in 500 ml **Fix Solution** with gentle agitation OVERNIGHT.
2. Pour off **the Fix Solution** and replace with 500 ml **Wash Solution**.
3. Agitate gently for 30 minutes.
4. Replace with 250 ml of MQWater.

5. Remove Deep Purple from freezer; allow to stand at RT for 10 minutes before opening.
6. Shake bottle of Deep Purple well.
7. Add 1.25 ml Deep Purple to the water.
8. Incubate with gentle agitation for 1 hour; **KEEP IN DARK**.
9. Pour off stain (do not use again).
10. Replace with **Storage Solution**.
11. Cover and store in a dark place until imaged.

Procedure for Non-Backed gels:

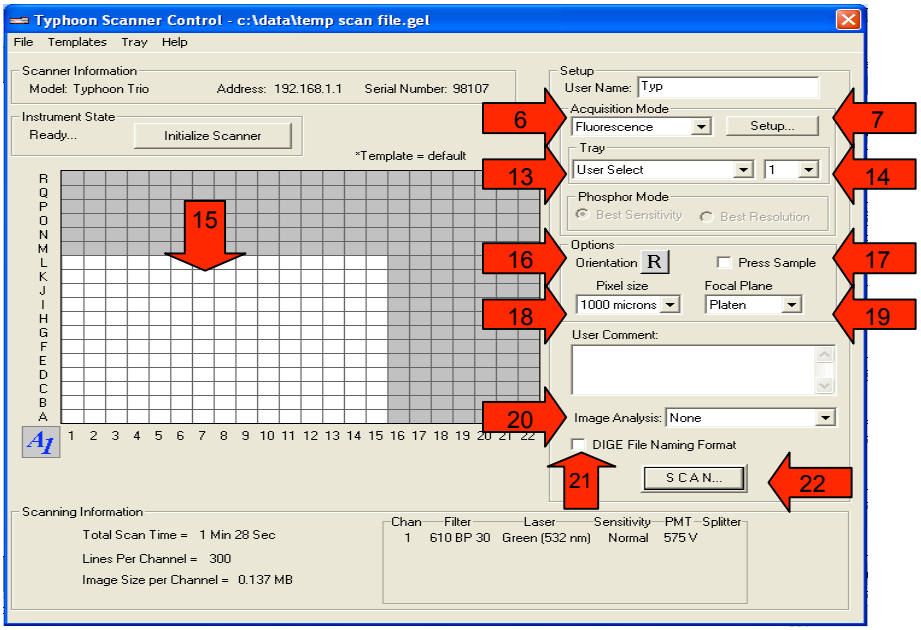
Mini-gels:

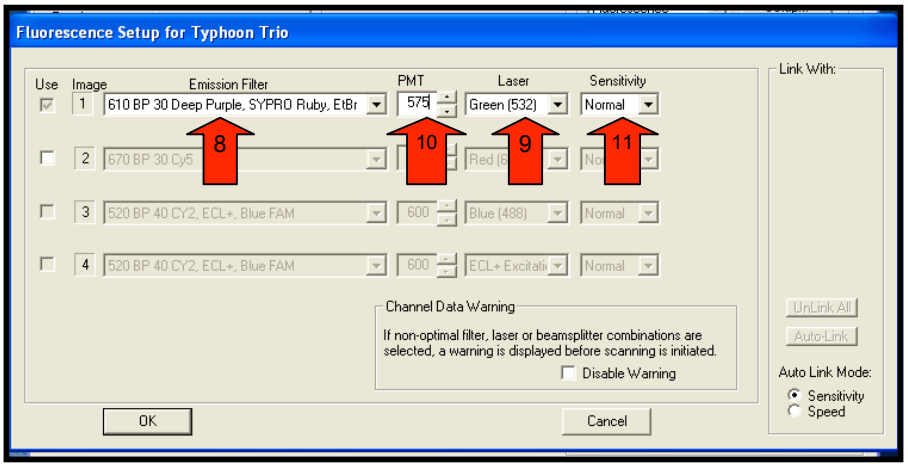
Use 100 mll fix and wash solutions.
Use 50 ml staining solution.

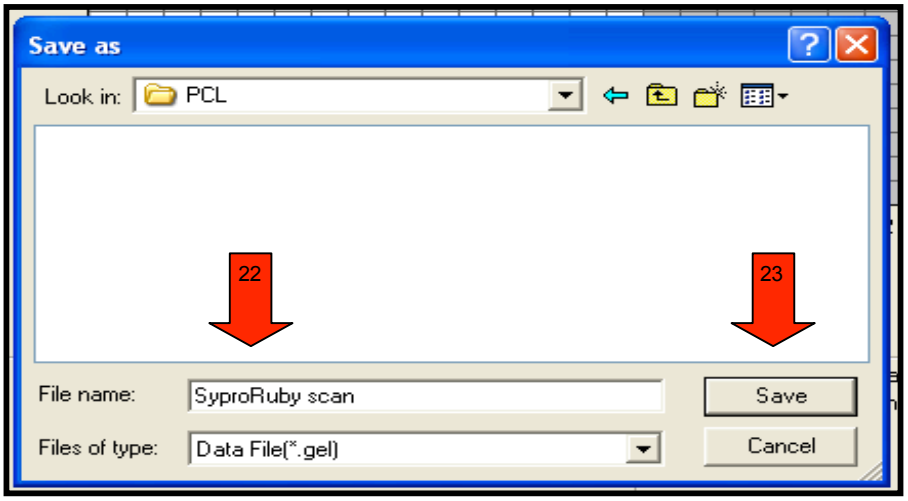
1. Incubate backed gel in 100 ml **Fix Solution** with gentle agitation OVERNIGHT.
2. Pour off **the Fix Solution** and replace with 100 ml **Wash Solution**.
3. Agitate gently for 30 minutes.
4. Replace with 50 ml of MQWater.
5. Remove Deep Purple from freezer; allow to stand at RT for 10 minutes before opening.
6. Shake bottle of Deep Purple well.
7. Add 0.25 ml Deep Purple to the water.
8. Incubate with gentle agitation for 1 hour; **KEEP IN DARK**.
9. Pour off stain (do not use again).
10. Rinse with **Storage Solution** 2 x 15 minutes.
11. Cover and store in a dark place until imaged.
12. Rinse gel 1 min with mQWater before imaging.
13. Image on non-fluorescent glass plate; set imager platen for +3 mm.

SCANNING SYPRORUBY™ AND DEEP PURPLE™ STAINED GELS ON TYPHOON TRIO

1. Open lid
2. Place gel on platen; do not cover with plastic wrap or glass
    a. WARNING: BE SURE THAT GEL IS WASHED IN WATER FOR A MINIMUM OF 10 MINUTES BEFORE PLACING ON THE PLATEN
3. Note coordinates on rulers to locate the gel
4. Close lid
5. Launch Typhoon Scanner software from the desktop
6. Set *Acquisition Mode* for *Fluorescence* on pulldown menu
7. Click *Set Up*
8. Choose *SyproRuby, Deep Purple emission filter* from pulldown menu
9. Choose *532 nm (green) laser*
10. *Set* PMT at 575 Volts for initial scan (can be adjusted accordingly)
11. Set *Sensitivity* to *Normal*
12. Close window
13. Set *Tray* to *User Select* with pulldown menu
14. Select the number of gels you will be scanning at one time with the pulldown menu
15. Set coordinates for scanning on screen by click-n-drag of cursor on the platen
16. Select *Orientation* for “R”
17. Unclick *Press Sample*
18. Set *Pixel Size* at 1000 microns (test scan only)
19. Set *Focal Plane* to *Platen* on pulldown menu
20. Set *Image Analysis* to none with pulldown menu
21. Unclick *DIGE File Naming Format*
22. Click *Scan*
23. Enter Filename and location for file storage (Type of file = *.gel)
24. Save




Fluorescence Setup for Typhoon Trio
Use
Image
Emission Filter
PMT
Laser
Sensitivity
1
610 BP 30 Deep Purple, SYPRO Ruby, EtBr
575
Green (532)
Normal
2
670 BP 30 Cy5
3
520 BP 40 CY2, ECL+, Blue FAM
600
Blue (488)
Normal
4
520 BP 40 CY2, ECL+, Blue FAM
600
Normal
8
10
9
11
Link With:
UnLink All
Auto-Link
Auto Link Mode:
Sensitivity
Speed
Channel Data Warning
If non-optimal filter, laser or beamsplitter combinations are selected, a warning is displayed before scanning is initiated.
Disable Warning
OK
Cancel


Save as
Look in:
PCL
22
23
File name:
SyproRuby scan
Save
Files of type:
Data File(*.gel)
Cancel