SYPRO® Ruby Staining of ONCYTE® Porous Nitrocellulose Film Slides

1. Purpose

1.1. General staining of proteins deposited on porous nitrocellulose films for sample protein quantification.

2. Equipment & Materials

2.1. Equipment

2.1.1. Orbital Shaker

2.1.2. Microarray Fluorescence Scanner such as the GenePix 4000B. Microarray Scanner (Molecular Devices)

2.2. Materials

2.2.1. Glass staining dishes with lids (Wheaton Cat# 900203).

2.2.2. Glass slide racks with handles (Wheaton Cat# 900204, 900205).

2.2.3. ProPlate™ modules (Grace Bio-Labs)

2.3. Reagents

2.3.1. SYPRO® Ruby (Invitrogen Cat# S12000)

2.3.2. Super G Blocking Reagent, optional (Grace Bio-Labs)

2.3.3. Fixative Solution: 7% acetic acid, 10% methanol in distilled water

2.3.4. Distilled water

3. Procedure

3.1. Optional: Submerge spotted protein array in Super G blocking reagent for 1 hour with no agitation.

3.1.1. Use of Super G blocking reagent will provide lower background fluorescence levels.

3.2. Transfer slides to a staining jar containing fresh water.

3.2.1. Wash 3 times for 15 minutes each, with agitation (105 rpm on orbital shaker).

3.3. Transfer slides to a staining jar containing the fixative solution.

3.3.1. Incubate for 15 minutes, with agitation (105 rpm on orbital shaker).

3.4. Transfer slides to a staining jar containing fresh water.

3.4.1. Wash 4 times for 5 minutes each, with agitation (105 rpm on orbital shaker).

3.5. Transfer slides to a staining jar containing the SYPRO® Ruby stain.

3.5.1. Alternatively: To save on reagent, instead of a staining jar use a ProPlate™ module such as the 1-well module.

3.5.2. Apply a 1-well ProPlate™ module to the wet slide (work quickly to prevent slide drying) and add 2 ml of SYPRO® Ruby stain.

3.6. Perform staining for 30 minutes at room temperature, with agitation (105 rpm on orbital shaker).

3.6.1. If using a ProPlate™ module, perform incubation with agitation at 50 rpm.

3.7. Transfer slides to a staining jar containing fresh water.
  3.7.1. Wash 4 times for 1 minute each, with agitation (105 rpm on orbital shaker).
  3.7.2. If using a ProPlate™ module, remove SYPRO® Ruby from chamber with a pipette and replace with 2 ml fresh water. Perform the first water wash in the ProPlate™ module, then unclip and transfer slide to a staining jar for washes 2-4.
3.8. Air-dry the slides in the dark.
3.9. Scan slides using a fluorescent scanner (these settings serve as a starting point and may vary from scanner to scanner):
  3.9.1. Axon 4000B Scanner
    3.9.1.1. 532 nm: 33% laser power, 400 PMT setting
    3.9.1.2. 635 nm: 100% laser power, 800 PMT setting

See Appendix for typical results obtained with this method

4. References

1. SYPRO® Ruby Protein Gel Stain Product Information, Molecular Probes, Rev. 11/20/2007.
2. SYPRO® Ruby Protein Stains Instruction Manual, BioRad, 4006173 Rev B.

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Appendix

Figure 1. SYPRO® Ruby Protein Quantification on ONCYTE® Film Slides.

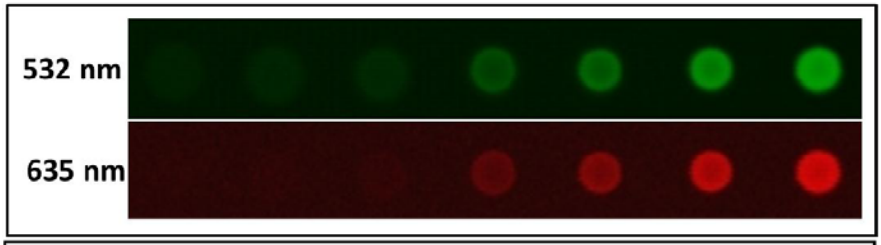

Figure 1. Total protein may be quantified after spotting on ONCYTE® Film Slides by staining with SYPRO® Ruby. Shown is a range of standard protein (BSA) at spotting concentrations ranging from 0.01 – 5 mg/ml after on-slide staining with SYPRO® Ruby. Fluorescence was quantified using two common excitation wavelengths (532 and 635 nm) and was obtained with an Axon 4000B microarray scanner.

Figure 2. Fluorescent Signal from SYPRO® Ruby Stained Protein on ONCYTE® Film Slides.

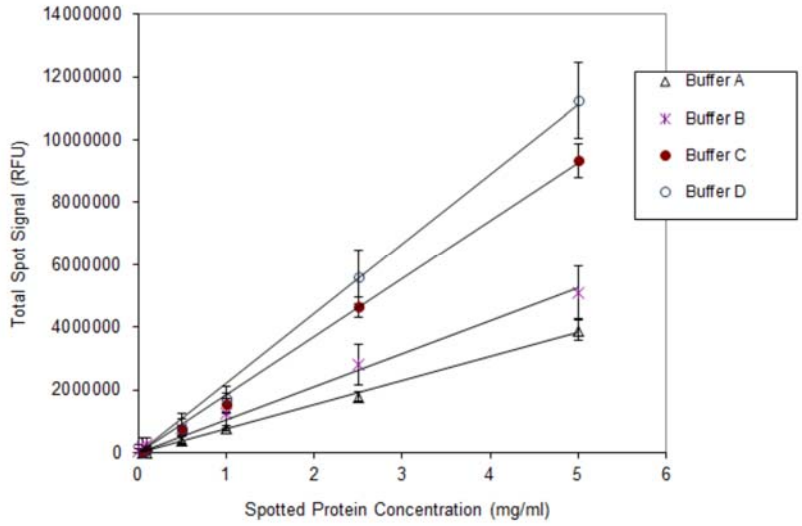

Figure 2. Fluorescent signal after staining with SYPRO® Ruby is linear over 3 orders of magnitude. Shown is the dynamic range of protein staining with SYPRO® Ruby on a SuperNOVA nitrocellulose film slide blocked with Super G blocking reagent. BSA standard was spotted in various RPPA lysate buffers with R^2 averaging 0.9955 for all of the buffers (Buffer A: SDS/Tris, Buffer B: SDS/Tris/Triton; Buffer C: Urea; Buffer D: Grace Bio-Labs RPPA lysis buffer).