Fast Green Staining of ONCYTE® Porous Nitrocellulose Film Slides with near IR Detection

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. ArrayCAM™ Microarray Imager (Grace Bio-Labs)

2.2. Materials and Reagents

- 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)
- 2.2.4. Fast Green FCF (Sigma-Aldrich Cat#F7258)
- 2.2.5. Methanol
- 2.2.6. Glacial Acetic Acid
- 2.2.7. Distilled Water

2.3. Solutions

- 2.3.1. De-Staining Solution (1000mL):
  - 2.3.1.1. Methanol (300mL, 30%)
  - 2.3.1.2. Glacial Acetic Acid (70mL, 7%)
  - 2.3.1.3. Water (630mL, 63%)
- 2.3.2. Fast Green Stock Solution (400x, 10mL)
  - 2.3.2.1. Fast Green (0.1g)
  - 2.3.2.2. De-Staining Solution (9.9mL)
- 2.3.3. Fast Green Staining Solution (1x, 1000mL)
  - 2.3.3.1. Fast Green Stock Solution (2.5mL)
  - 2.3.3.2. De-Staining Solution (997.5mL)
- 2.3.4. 1% NaOH

3. Procedure

3.1. Transfer slides to a staining jar containing fresh distilled water.
- 3.1.1. Wash for 5 minutes with agitation (105 rpm on orbital shaker).

3.2. Transfer slides to a staining jar containing 1% NaOH.
- 3.2.1. Incubate for 15 minutes with agitation (105 rpm on orbital shaker).

3.3. Transfer slides to a staining jar containing fresh distilled water.
- 3.3.1. Rinse briefly by submerging slides repeatedly 10-20 times over 1 minute.

3.4. Transfer slides to a staining jar containing fresh distilled water.
- 3.4.1. Wash for 10 minutes with agitation (105 rpm on orbital shaker).

3.5. Transfer slides to a staining jar containing De-Staining Solution.
- 3.5.1. Wash for 15 minutes with agitation (105 rpm on orbital shaker).

3.6. Transfer slides to a staining jar containing Fast Green Staining Solution
  3.6.1. Wash for 3 minutes with agitation (105 rpm on orbital shaker).
3.7. Transfer slides to a staining jar containing fresh water.
  3.7.1. Rinse briefly by submerging slides repeatedly 10-20 times over 1 minute.
3.8. Transfer slides to a staining jar containing De-Staining Solution.
  3.8.1. Wash for 15 minutes with agitation (105 rpm on orbital shaker)
3.9. Transfer slides to a staining jar containing fresh water.
  3.9.1. Rinse briefly by submerging slides repeatedly 10-20 times over 1 minute.
3.10. Dry the slide by centrifugation.
3.11. Scan slides using a fluorescent imager capable of measuring at 800 nm (settings and results may vary with imaging system and filters employed):
  3.11.1. ArrayCAM Microarray Imager
    3.11.1.1. 800 nm bandpass
    3.11.1.2. Typical Settings:
      - Exposure: 200 msec.
      - Acquisition Time: 4 sec.
      - Gain: 20%

See Appendix for typical results obtained with this method.

4. References

1. Loebke, et al. (2007) Infrared-based protein detection arrays for quantitative proteomics. Proteomics 7, 558-584.
2. Levine, et al. (2006) Quantitation of protein on gels and blots by infrared fluorescence of Coomasie blue and Fast Green. Analytical Biochemistry 350, 233-238.

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Appendix

Figure 1. Fast Green Protein Quantification on ONCYTE® Film Slides with near IR detection using ArrayCAM.

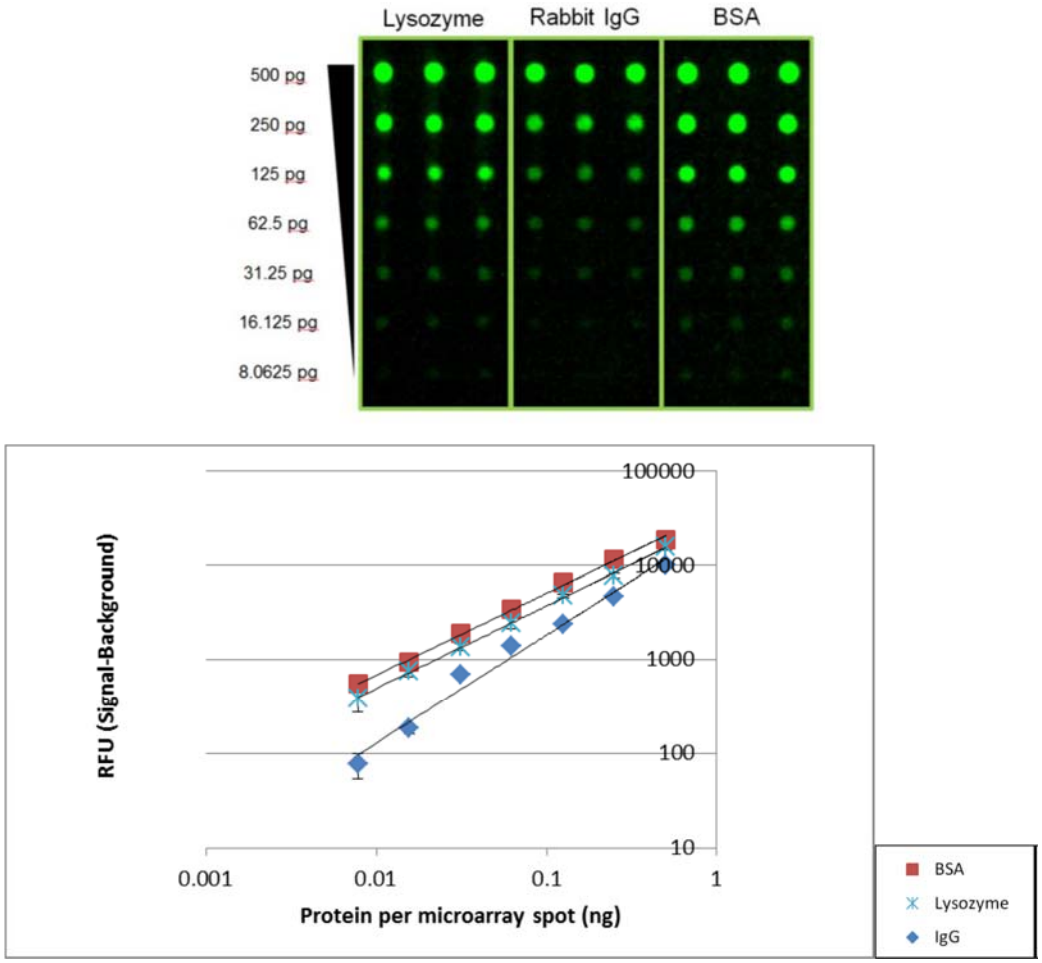

Figure 1. Total protein may be quantified after spotting on ONCYTE® AVID Film Slides by staining with Fast Green and measuring fluorescence using an ArrayCAM, an imaging system developed at Grace Bio-Labs. ArrayCAM is a CCD-based imaging system using laser excitation at 405 nm. Fast Green emission is 800 nm. (NOTE: Similar results were obtained with ONCYTE® SuperNOVA Film Slides from Grace Bio-Labs.)

A. Shown is a range of standard proteins from 8 – 500 pg after on-slide staining with Fast Green.
B. Detection and quantitation of BSA, Lysozyme, and IgG using a Fast Green staining protocol (see attachment) with ArrayCAM are linear down to the lowest protein deposition (8 pg).

Figure 2. Quantitation of Cell Lysates on RPPA using Fast Green with ArrayCAM produces results comparable to Sypro Ruby Protein Stain measured on a GenePix scanner (Molecular Devices, Sunnyvale, CA).

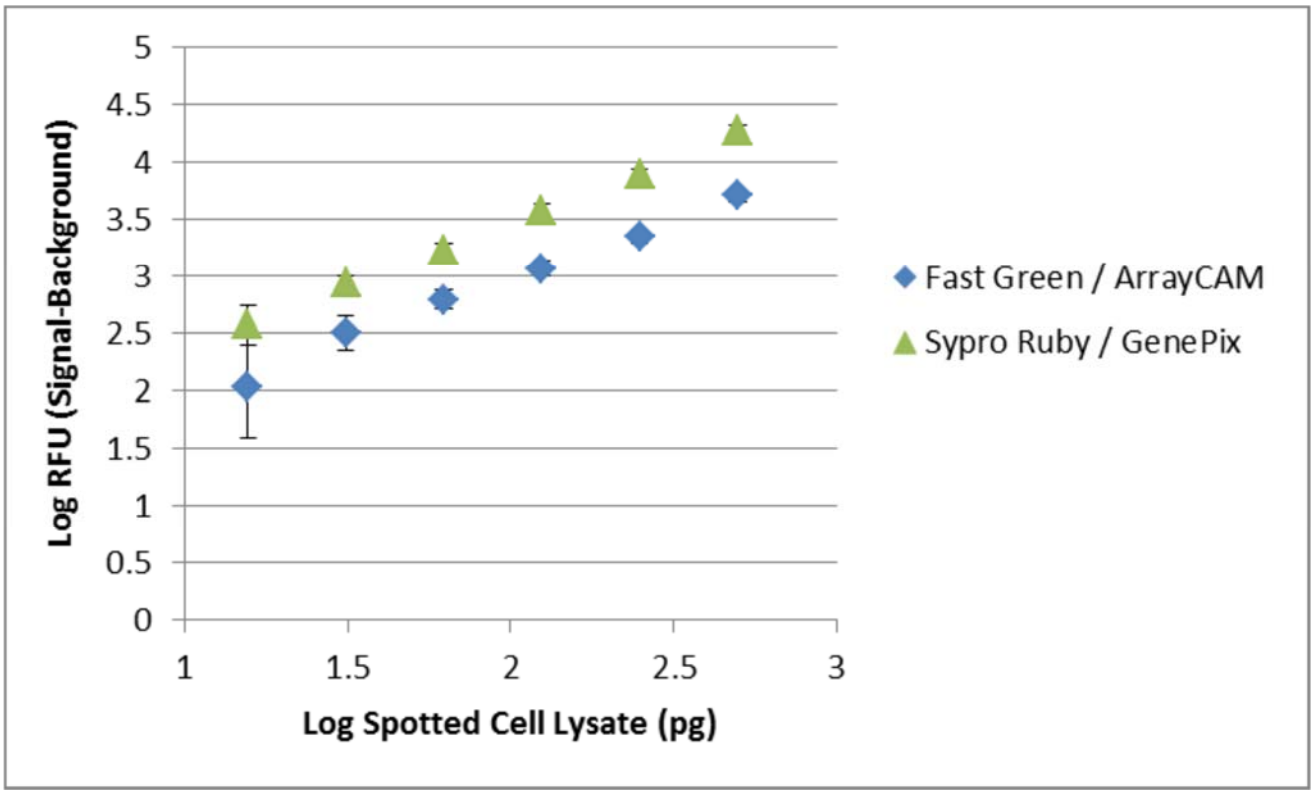

Figure 2. Fluorescent signal for total protein detection with Fast Green/ArrayCAM yields linear results at cell lysate concentrations commonly used for RPPA analysis down to approximately 8 pg protein per spot. Similar results were obtained using a standard protein stain method (SYPRO® Ruby total protein stain; 532nm/575nm detection) with the use of a focused-laser microarray scanner (Molecular Devices GenePix 4400). Cell lysates were from Calyculin A-treated Jurkat cells spotted in 2-fold serial dilution starting with a concentration of 1 mg/ml in a Tris/SDS/Glycerol lysis buffer.