Syto® 60 Staining of Nucleic Acids in Gels

Developed for

Aerius, and Odyssey® Family of Imagers

LI-COR®

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The Syto® 60 stain is a red fluorescent nucleic acid stain supplied as a 5 mM solution in DMSO by Invitrogen, P/N S-11342. Any questions regarding the Syto 60 stain should be directed to Invitrogen (www.invitrogen.com).

I. INTRODUCTION

Invitrogen's patented Syto dyes are cell-permeant cyanine dyes that bind to nucleic acids. Several Syto dyes are available with varying cell permeability, fluorescence enhancement upon binding to nucleic acids, excitation and emission spectra, and nucleic acid selectivity and binding affinity. The Syto 60 stain has absorption and fluorescence emission maxima of 652/678 nm. Nucleic acids stained with the Syto 60 stain can be detected and quantified on the Odyssey® Infrared and Odyssey Fc Imaging Systems using the 700 nm channel.

In the procedures outlined, the Syto 60 dye was used to stain serial dilutions of a 1 kb DNA ladder and a 50 bp DNA ladder (New England Biolabs, P/N N3232 and N3236, respectively). Three methods are presented for staining of DNA in this technical note. The Syto 60 stain can be included in the DNA sample for detection using an Odyssey Imaging system. The Syto 60 stain can also be combined with ethidium bromide (EtBr) and included in the DNA sample for visualization on an Odyssey Imaging System and on a UV transilluminator; or the Syto 60 stain can be diluted and used alone as a post-electrophoresis gel stain. For additional information, see *Imaging Nucleic Acid Gels on the Odyssey Fc Imager*.

II. METHODS

Method I. Electrophoretic Staining

Purpose: To obtain an archivable, digital image of a DNA agarose gel using an Odyssey Imaging System.
NOTE: This method may not be optimal for visualizing bands smaller than 100 bp.

Method:

1. Dilute the Syto 60 stain 1:1000 in TE buffer, mix well.
   NOTE: Syto 60 stain is stable for up to 1 week at 4 °C when diluted.
2. Prepare DNA samples in loading dye and reserve an additional 1 µL in the final volume to accommodate the 1 µL of Syto 60 stain for loading.
3. To each sample, add 1 µL of the diluted Syto 60 stain and mix well with a pipettor.
4. Incubate at room temperature for 5 minutes.
5. Load the samples on the gel.
6. Run the gel at ~5-10 V/cm for ~1 hour or less.

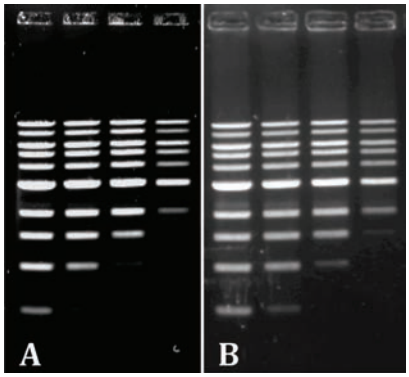

Figure 1. Two-fold dilutions of 1 kb ladder, from 1 µg to 0.125 µg, separated on a 1.2% agarose gel at 8V/cm in 1X TAE buffer for 1 hour. Panel A is the image of the gel obtained from the Odyssey Infrared Imaging System using an intensity of 5.0, gel face down. Panel B is the image of the gel acquired for 2 minutes using the Odyssey Fc Imaging System 700 nm channel, gel face up.

7. Use an Odyssey®, Odyssey CLx, Odyssey Sa, Odyssey Fc, or Aerius Imaging System to obtain a digital image of the Syto 60-stained DNA.

   Odyssey or Odyssey CLx System Settings:
   - Gel face down on scan bed
   - 700 nm channel intensity: 5-8
   - Focus offset: 0.5 mm

   Odyssey Sa or Aerius System Settings:
   - Gel face down on scan bed
   - 700 nm channel intensity: 5-8
   - Focus offset: 1.7 - 2.0 mm

   Odyssey Fc System Settings:
   - Gel face up on imaging tray
   - Acquisition time: 2 min.

Method II. Dual Electrophoretic Staining

Purpose: To obtain a digital image using an Odyssey® Imaging System and then visualize DNA bands on a UV transilluminator for excision.
NOTE: This method may not be optimal for visualizing bands smaller than 100 bp.

Method:

1. Dilute the Syto 60 stain 1:1000 in TE buffer, mix well.
   NOTE: The Syto 60 stain is stable for up to 1 week at 4 °C when diluted.
2. Dilute EtBr (10 mg/mL solution) 1:500 in TE buffer, mix well (made fresh).
3. Prepare DNA samples in loading dye and reserve an additional volume of 2 µL to accommodate the volume of Syto 60 stain and EtBr for loading.
4. To each sample, add 1 µL of the diluted EtBr and mix with a pipettor.
5. To each sample, add 1 µL of the diluted Syto 60 stain and mix with pipettor.
6. Incubate at room temperature for 5 minutes.
7. Load the samples.
8. Run the gel at ~5-10 V/cm for ~1 hour or less.
   NOTE: Longer run times result in fading of the Syto 60 intensity.
9. Image on an Odyssey Imaging System in the 700 nm channel to obtain a digital image of Syto 60-stained DNA.

   Odyssey or Odyssey CLx Imaging System Settings:
   - Gel face down on scan bed
   - 700 nm channel intensity: 5-8
   - Focus offset: 0.5 mm

   Odyssey Sa or Aerius System Settings:
   - Gel face down on scan bed
   - 700 nm channel intensity: 5-8
   - Focus offset: 1.7 - 2.0 mm

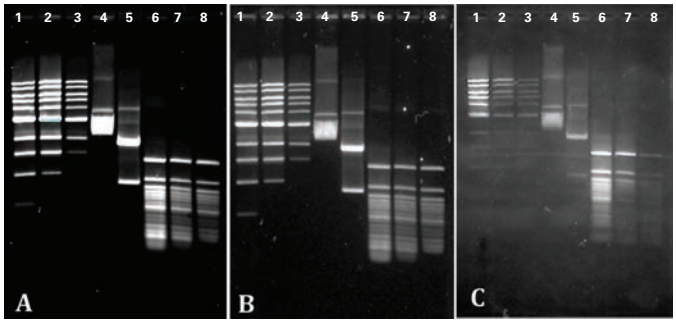

Figure 2. A 1.2% agarose gel was imaged using the Odyssey® Infrared Imaging System (panel A), Odyssey Fc Imaging System (panel B) or a UV transilluminator and the image captured using Polaroid 667 film (panel C). Lane 1) 1 µg 1 kb ladder; Lane 2) 0.5 µg 1 kb ladder; Lane 3) 0.25 µg 1 kb ladder; Lane 4) 0.5 µg pUC 19; Lane 5) 0.5 µg pUC19/HindIII / XmnI; Lane 6) 1 µg 50 bp ladder; Lane 7) 0.5 µg 50 bp ladder; Lane 8) 0.25 µg 50 bp ladder. The gel was electrophoresed for 8 V/cm in 1X TAE buffer for 1 hr. The Odyssey intensity setting for the 700 nm channel was 8 and focus offset was 0.5 with the gel face down. The Odyssey Fc acquisition was 2 minutes, gel face up.

Odyssey Fc System Settings:
- Gel face up on imaging tray
- Acquisition time: 2 min.

UV Transilluminator:
- Place gel on UV transilluminator to identify bands for excision. If the band(s) to be excised are not bright enough, the gel can be soaked for a short time in a 2 mg/mL solution of EtBr in TAE or TBE buffer after imaging on an Odyssey System.

Hints and Tips for Methods I and II

1. The range of dilution for the Syto 60 stain is 1:500 to 1:20,000. The dilution to use is dependent on the DNA size, concentration, and whether the Syto 60 stain will be used in combination with EtBr.
   NOTE: This method may not be optimal for visualizing bands smaller than 100 bp.
2. The Syto 60 stain, diluted within the recommended range in TE buffer, is stable for 1 week at 4 °C.
3. EtBr is not stable in TE and should be diluted fresh each time.
4. The grade of agarose is important. High grade or Molecular Biology grade agarose is less likely to cause "speckling" on Odyssey images.
5. When using the Odyssey, Odyssey CLx, Odyssey Sa, and Aerius to image DNA gels stained with Syto 60 stain, it may be necessary to scan the gel with the front side on the glass and/or adjust the focus offset, depending on the gel thickness. A 5 mm - 7 mm thick gel is optimum.
6. Addition of EtBr to the gel and running buffer with the Syto 60 stain added in the sample is not recommended.

Method III. Post-electrophoretic staining

Purpose: To obtain an archivable, digital image of a DNA agarose gel using an Odyssey Imaging System.
NOTE: This method IS recommended for visualizing <100 bp.

Method:
1. Two parallel 1.3% agarose/TBE gels were loaded with serial two-fold dilutions of 100 bp DNA ladder (New England Biolabs) from 1 µg to 0.3 µg per lane.
2. The gels were electrophoresed in 1X TBE running buffer at approximately 5 V/cm.

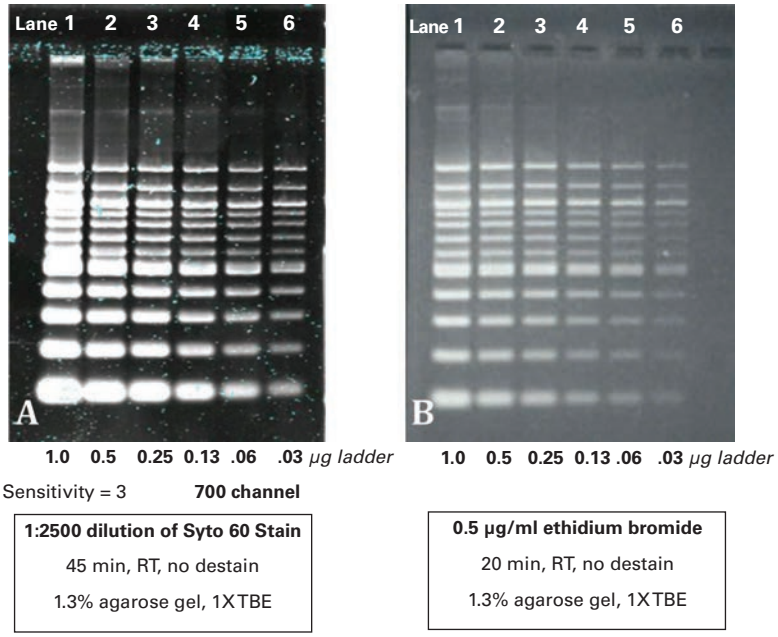

Figure 3. Panel A: Image of a Syto 60 stained gel using the Odyssey® Infrared Imaging System. Panel B: Image of ethidium bromide-stained gel using a Polaroid camera.

3. One gel was stained with Syto 60 dye diluted 1:2500 in water for 45 minutes at room temperature, rinsed briefly with double distilled water and then imaged in the 700 nm channel using an Odyssey Family Imager, or Aerius. Use the instrument settings provided in Methods I and II.

4. The other gel was stained in 0.5 µg/mL ethidium bromide for 20 minutes at room temperature, rinsed briefly in water, and imaged using a UV transilluminator and a standard CCD camera. The Odyssey Fc with 600-channel capabilities can also be used to image ethidium bromide gels. See *Imaging Nucleic Acid Gels on the Odyssey Fc Imager* for additional information.

Recommended Dilutions and Time Requirements for Method III.

Gel Conditions: A 10 x 10 cm agarose gel, 5-8 mm thick, made with high-grade or molecular biology-grade agarose in 1X TAE or TBE buffer

The quickest staining time was 5 minutes using 1:2000 dilution of the Syto 60 stain in water. Gels were stained sufficiently in 15 minutes using a 1:2500 dilution. A 1:5000 dilution of Syto 60 stain requires at least 30-45 minutes of staining. The most dilute solution tested was 1:20,000 and the gel was stained sufficiently after 45 minutes. There was no significant improvement in sensitivity from 60 to 120 minutes using 1:10,000, 1:15,000 and 1:20,000 dilutions.

Syto® 60 Nucleic Acid Stain Dilution	Minimum Staining Time
1:2000	5-15 min
1:2500	15-30 min
1:5000	30-45 min
1:10000	45 min
1:15000	45 min
1:20000	45 min

Speckle Reduction

The appearance of speckles on the gel may be present after post-electrophoretic staining. Use the Odyssey Application software's "FILTER" then "Noise Removal" function, or Image Studio's "NOISE REDUCTION" function, to improve the appearance

of the images (see Figure 4). To reduce the appearance of speckles on the gel, cut off the wells before post-electrophoretic staining and rinse the gel in water.
NOTE: The type and concentration of agarose will affect the degree of speckling. For example, low melting-point agarose tends to be highly prone to speckling.

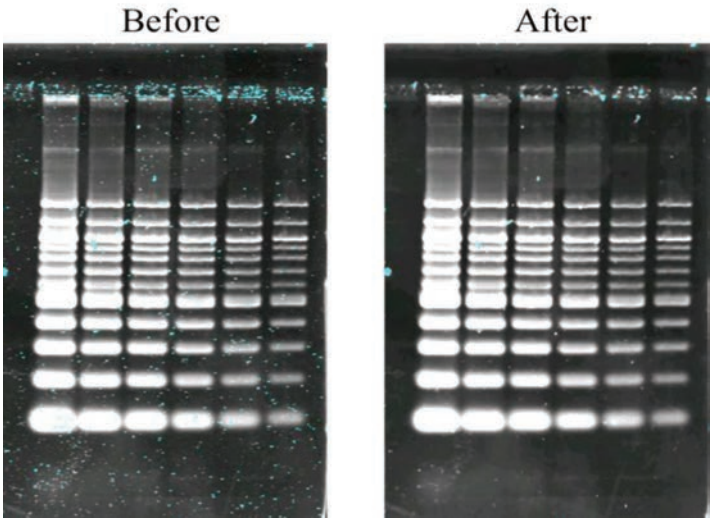

Figure 4. Image of agarose gel showing before and after using Odyssey® Infrared Imaging System software's "FILTER" then "Noise Removal" function.

III. CONCLUSIONS

A table of cost comparisons for the Syto 60 stain and the ethidium bromide staining reagents used for each method is provided below. The recommended dilution of the Syto 60 staining reagent makes it more competitive with ethidium bromide on a cost basis, and the small amount of Syto 60 stain used in the sample is environmentally friendly.

Cost Comparisons

	Dilution	Staining Method	Cost
Syto 60 stain	1:1000	Method I or II	$0.006 (1 µL/well, 8 wells)
Syto 60 stain	1:20000	Method I	$0.0003 (1 µL/well, 8 wells)
Syto 60 stain	1:2500	Method III	$7.56 (25 mL)
EtBr	1:500	Method II	$0.00006 (1 µL/well, 8 wells)
EtBr	1:2000	Method III	$0.049 (25 mL)

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