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Syto® 60 Staining of Nucleic Acids in Gels 🖘

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ABSTRACT

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.

EXTERNAL LINK

https://www.licor.com/documents/eo6n96n0kg8hfgc50dutu73byr0heg4r

techNote_Odyssey_Syt o60Staining_0816_988 12449 (1).pdf

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDFLINES

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

✓ protocols.io 09/28/2018 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.

See 'STEPS' for method.

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.

See 'STEPS' for method.

Odyssey or Odyssey CLx Imaging System Settings: Gel face down on scan bed

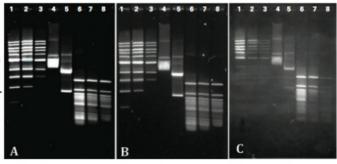
700 nm channel intensity: 5-8 • Focus offset: 0.5 mm Odyssey Sa or Aerius System Settings:

Gelface 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 / Xmnl; 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



1XTAE 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 inTAE orTBE buffer after imaging on an Odyssey System.

Hints and Tips for Methods I and II

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,

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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.

The Syto 60 stain, diluted within the recommended range in TE buffer, is stable for 1 week at 4 °C.

EtBr is not stable in TE and should be diluted fresh each time.

The grade of agarose is important. High grade or Molecular Biology grade agarose is less likely to cause "speckling" on Odyssey images.

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.

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.

See 'STEPS' for method.

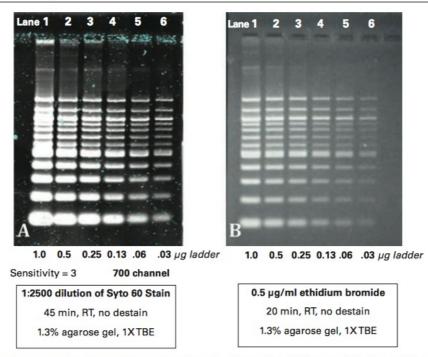


Figure 3. Panel A: Image of a Syto 60 stained gel using the Odyssey® Infrared Imaging System. Panel B: Image of ethidium bromide-stained gel using a Polaroid camera.

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	Minimum	
Nucleic Acid	Staining Time	
Stain		
Dilution		
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 'FILT ER" then "Noise Removal' function, or Image Studio's "NOISE REDUCT ION" 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.

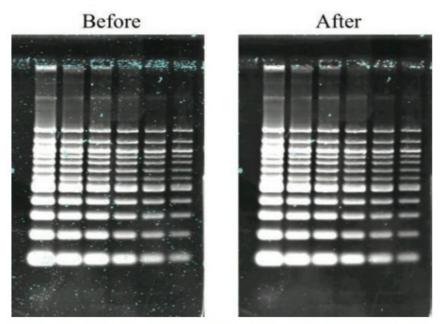


Figure 4. Image of agarose gel showing before and after using Odyssey® Infrared Imaging System software's "FILTER" then "Noise Removal" function.

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)	

SAFETY WARNINGS

See SDS (Safety Data Sheet) for hazards and safety warnings.

Method I. Electrophoretic Staining

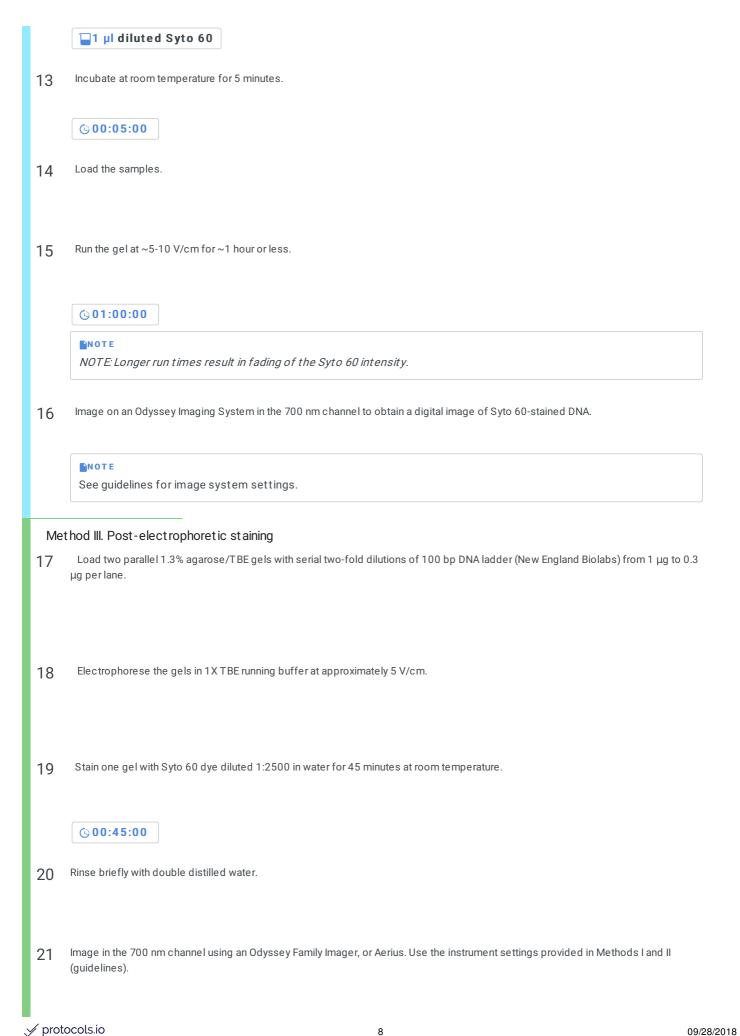
1 Dilute the Syto 60 stain 1:1000 in TE buffer, mix well.

NOTE

NOTE: Syto 60 stain is stable for up to 1 week at 4 $^{\circ}\text{C}$ when diluted.

Prepare DNA samples in loading dye and reserve an additional 1 μ L in the final volume to accommodate the 1 μ L of Syto 60 stain for loading.

3 To each sample, add 1 μ L of the diluted Syto 60 stain and mix well with a pipettor. ■1 µl diluted Syto 60 Incubate at room temperature for 5 minutes. 4 **©00:05:00** 5 Load the samples on the gel. 6 Run the gel at \sim 5-10 V/cm for \sim 1 hour or less. **(4) 01:00:00** 7 Use an Odyssey®, Odyssey CLx, Odyssey Sa, Odyssey Fc, or Aerius Imaging System to obtain a digital image of the Syto 60-stained **NOTE** See Guidelines for settings. Method II. Dual Electrophoretic Staining Dilute the Syto 60 stain 1:1000 in TE buffer, mix well. NOTE NOTE: The Syto 60 stain is stable for up to 1 week at 4 °C when diluted. Dilute EtBr (10 mg/mL solution) 1:500 inTE buffer, mix well (made fresh). 9 Prepare DNA samples in loading dye and reserve an additional volume of 2 µL to accommodate the volume of Syto 60 stain and EtBr 10 for loading. To each sample, add 1 μ L of the diluted EtBr and mix with a pipettor. 11 ■1 µl diluted EtBr To each sample, add 1 μL of the diluted Syto 60 stain and mix with pipettor. 12



22 Stain the other gel with 0.5 $\mu g/mL$ ethidium bromide for 20 minutes at room temperat	ture.
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©00:20:00

- 23 Rinse briefly in water.
- 24 Image using a UV transilluminator and a standard CCD camera.

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