Suggested Protocols for Working with Sybr® Green I :

(A) Using Sybr Green I (concentrated stock at 10,000X) to cast agarose gel with 1x Sybr Green I:

(This method gives fast results, but the stain in gel may affect the migration of DNA fragments.)

For example:

- Prepare 40 ml of molten agarose gel solution. Cool to about 60 ^{o}C. (Use a polypropylene container. Sybr Green I will be adsorbed onto the glass surface.)
- Add 4 µl 10,000X of concentrated Sybr Green I to the gel solution, mix thoroughly.
- Cast the gel as usual. Wait 45 to 60 minutes or until the gel solidifies and is ready for use.
- Load and run your DNA samples as usual.
- After electrophoresis, take the gel out of the warm buffer and let it cool down for a while; visualize the DNA bands with the PrepOne™ Sapphire.

Tips for using Sybr Green I to prepare precast agarose gels:
(1) Sybr Green I is sensitive to heat. Do not heat Sybr Green I in the microwave.
Boiling & near boiling temperatures destroy the Sybr Green I's ability to stain nucleic acid.
(2) Dilute Sybr Green I stock reagent (10,000X) 1: 10,000 into the gel solution right before pouring the gel. Cool the gel solution to ~ 60^{o}C or below.
For example: add 2 µl Sybr Green I to 20 ml gel solution, mix well before pouring the gel.
(3) Use polypropylene containers to prepare the gel solution mixture. Sybr Green I binds to glass and other non-polypropylene plastics which may result in a decreased sensitivity from your sample.

(B) Post staining DNA bands following gel electrophoresis with 3x Sybr Green I in running buffer:

(This is the best method for maximum sensitivity! DNA migration will not be affected by the stain.)

- Separate the DNA samples by standard electrophoresis.
- Prepare a staining buffer (**3X Sybr Green I** in running buffer) ---
  For example: add 12 µl of 10,000X Sybr Green I to 40 ml running buffer (1XTAE or 1XTBE, pH 7.5 to 8.0), mix thoroughly. It is very important to use polypropylene containers instead of glass containers. Glass surfaces will absorb Sybr Green and render it unable to staining the DNA.
- Place the gel into the staining buffer. Ensure there is enough buffer to cover the whole gel. Incubate for **at least 60 minutes with gentle agitation**. Cover the container with a piece of aluminum foil to prevent photo bleaching of the dye from the ambient light.