PRODUCT INFORMATION

Product Name : Gel Indicator™ DX
Code No. : DM580 *This product is research use only*

Kit Components:

Code No.	Components	Contents	Size
DM581	Four–Colors Indicator	Xylen cyanol FF, Cresol Red, BromoCresol Purple, Orange G, Sodium EDTA, Tris-HCl buffer, Glycerin, < DNase free >	1 ml
DM530	6×Orange Loading Dye	Orange G, Sodium EDTA, Glycerin, Tris-HCl buffer, < DNase free >	1 ml
DM540	Gel Indicator Solution DX	Dye, < DNase free >	1 ml × 3
DM585	GI Coloring Solution	Dye, < DNase free >	15 ml

Introduction:

Excising DNA from agarose gels is often used for DNA-cloning work. However conventional method of visualizing DNA with ethidium bromide and exposure UV light damages DNA and significantly decreases cloning efficiency (1, 2). By using the Gel Indicator™ DX, UV light is not needed to excise DNA from agarose gels. DNA in agarose gel is colorized by Gel Indicator Solution DX or GI Coloring Solution and can be excised under visible light. Higher cloning efficiency is found using by DNA prepared with the Gel Indicator™ DX than with ethidium bromide and UV light.

Gel Indicator™ DX has been improved on its sensitivity of DNA detection. With GI Coloring Solution, DNA band in agarose gel can be detected > 20 ng. The excised DNA with this kit can be purified by organic solvent extraction or with other commercial kits. (All tested commercial kits worked well.). DNA excised from agarose gel can be used many downstream experiments, such as enzyme digestion, PCR, sequencing, *in vitro* transcription, as well as cloning.

1. Cariello NF, Keohavong P, Sanderson BJ, Thilly WG., (1988) DNA damage produced by ethidium bromide staining and exposure to ultraviolet light, Nuc. Acids. Res., **16**, 4157.
2. Hartman P.S., (1991) Transillumination can profoundly reduce transformation frequencies. Biotechniques, **11**, 747-748.

Storage condition : Stable at 20° to 25°C for six months from the date of receipt.

Additional Materials Needed :

To excise DNA bands in agarose gel, the following materials are required.

- Agarose for DNA electrophoresis
- 0.5×TAE buffer or 1×TAE
- Microwave
- Electrophoresis apparatus
- Tray for staining agarose gel
- Razor blade

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Before start:

• Wear gloves and protecting clothing while handling this kit. The stain of Gel Indicator Solution DX or GI Coloring Solution is strong.

• DNA size should be 250 - 10, 000 bp for detection or separation by agarose gel. Use protocol II to detect DNA of 100-250 bp in size. DNA less than 100 bp is not recommended for the Gel Indicator™ DX.

• If DNA amount is more than a few µg to be excised, use Protocol I to save time. Protocol II can be applied without performing Protocol I and also after Protocol I.

Protocol I

Features:

- DNA can be excised just after electrophoresis.
- The Four–Colors Indicator let know approximate sizes of DNA
- DNA detection limit / band on agarose gel: 500 ng

1. Preparation of gel

To 100 ml of 0.5×TBE buffer, add 0.8 g of powdered agarose for DNA separation. Mix them and dissolve agarose in the microwave. Add 100 µl of Gel Indicator Solution DX to the agarose (100 ml) after removing from microwave. Mix them by swirling and pour the agarose into mold and set a comb. Allow the gel to harden.

2. Sample preparation

Dye in the Gel Indicator Solution DX has a lower sensitivity than ethidium bromide. DNA sample to be excised, restriction digest or PCR product, should be greater than 500 ng. Mix 10 µl of DNA Sample (>500 ng / band) and 2 µl of 6×Orange Loading Dye and load to a well. Load 10 µl of Four–Colors Indicator to a well for a reference indicator. Do not mix the Four–Colors Indicator and DNA samples. Dyes in Four–Colors Indicator migrate on 0.8 % agarose gel described in Table 1, although the relationships are affected by run time of electrophoresis.

3. Excising DNA

During electrophoresis DNA can be visible as a blue band. DNA can be excised from agarose gel under normal light with a razor blade.
If DNA band is hardly visible, and amount of DNA in the band is expected over 20 ng, proceed to Protocol II.

* Instead of 0.5×TBE, 1×TAE also can be used.

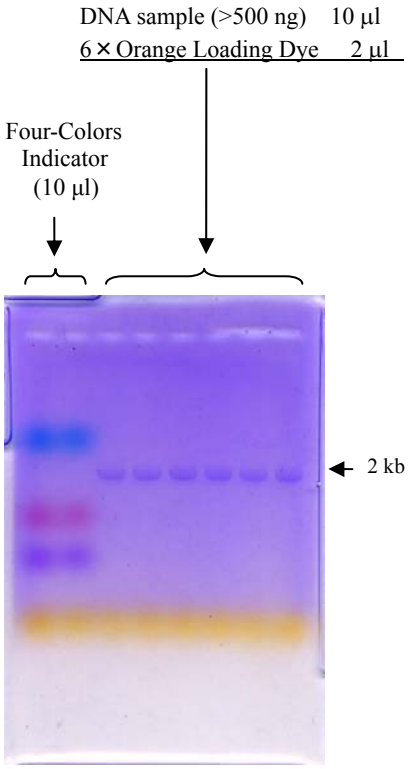

Fig. 1. Electrophoresis by Protocol I
Agarose Electrophoresis (0.8 %), 1×TAE

Table 1	0.5×TBE	1×TAE
Blue (Xylene Cyanol FF)	7000 - 6000 bp	7000- 6000 bp
Red (Cresol Red)	900 - 700 bp	1600-1300 bp
Purple (BromoCresol Purple)	300-500 bp	800-600 bp
Orange (Orange G)	50-100 bp	50-100 bp

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Protocol II

Features:

- After electrophoresis with or without Gel Indicator Solution DX, agarose gel is stained with diluted GI Coloring Solution
- DNA detection limit / band on agarose gel: >20 ng
- Recommended DNA size for excision: 100 - 10, 000 bp

1. Agarose gel and electrophoresis

Prepare 0.5-2.0 % agarose gel with 0.5×TBE* buffer. Gel concentration depends on DNA size to be separated. Set the agarose gel into an electrophoresis apparatus with 0.5×TBE* buffer. Mix 10 µl of DNA Sample and 2 µl of 6×Orange Loading Dye. Load the mixed sample to a well and start electrophoresis. Agarose gel after electrophoresis by using of the Gel Indicator Solution DX can be also used.

* Instead of 0.5×TBE, 1×TAE also can be used

2. Preparation of diluted GI Coloring Solution (0.2 %)

Mix one volume of GI Coloring Solution and 500 volume of pure water. For example, 200 µl of GI Coloring Solution should be mixed with 100 ml of pure water. Prepare enough 0.2 % GI Coloring Solution to immerse the agarose gel.

3. Staining with 0.2 % GI Coloring Solution

After electrophoresis, the agarose gel is transferred to a tray containing the 0.2 % GI Coloring Solution. Stain the agarose gel over 2 hr with gentle agitation. When DNA is visible as a blue band, DNA can be excised from agarose gel with a razor blade or gel-excision devices, for example, x-tracta (LabGadget, USA, Funakoshi, Japan).

‡ Reuse of diluted GI Coloring Solution is not recommended.

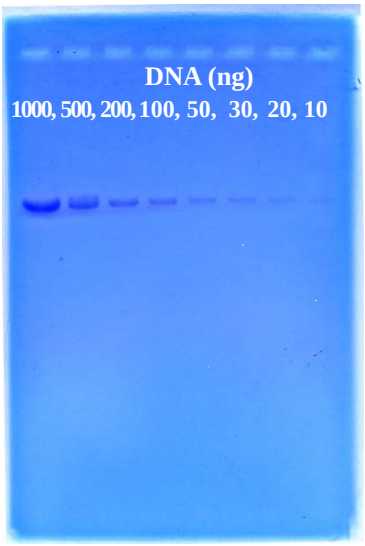

Fig. 2.
Detection of DNA by Protocol II.
Agarose Electrophoresis (0.8 %),

Ver. 1.2