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E-Gel™ 48-Well 2% Agarose Gels

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E-Gel™ *48* Gels are pre-cast, ready-to-use, 48-well agarose gels designed for medium-throughput resolution of DNA fragments. Each gel contains 48 sample wells and 4 marker wells in a 2% high-resolution agarose. These types of standardized gels can be ran on an E-Base platform and visualized using Gel Doc XR+.

MAN0001550_egel48_qrc.pdf

MAN0018775_epage_QR.pdf

Allyson Hirsch, George Testo 2022. E-Gel™ 48-Well 2% Agarose Gels . **protocols.io** https://protocols.io/view/e-gel-48-well-2-agarose-gels-cajsscne

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Reagents

- 1Kb Plus DNA Ladder

Supplies

- 96-well skirted plate(s)
- Green temporary seal(s)
- Foil seal(s)

Equipment

- 200uL multi-channel pipette & tips
- 20uL multi-channel pipette & tips
- 20uL pipette & tips

Ethidium bromide (EtBr) is commonly used as a non-radioactive marker for identifying and visualizing nucleic acid bands in electrophoresis and in other methods of nucleic acid separation. EtBr is a dark red, crystalline, non-volatile solid, moderately soluble in water, which fluoresces readily with a reddish-brown color when exposed to ultraviolet (UV) light. Although it is an effective tool, its hazardous properties require special safe handling and disposal procedures.

EtBr is a potent mutagen (may cause genetic damage), and moderately toxic after an acute exposure. EtBr can be absorbed through skin, so it is important to avoid any direct contact with the chemical. EtBr is an irritant to the skin, eyes, mouth, and upper respiratory tract. It should be stored away from strong oxidizing agents in a cool, dry place, and the container must be kept undamaged and tightly closed.

- Good work practices can help reduce hazardous exposures.
- To prevent inhalation exposure, work with EtBr powder or crystals in a fume hood, or work with premixed EtBr solutions or tablets to avoid handling the powder directly.
- To prevent skin contact when working with liquid solutions, wear protective gloves, a laboratory coat, and chemical goggles. Change gloves frequently.
- Provide EtBr users with safety training on EtBr hazards, use, and proper cleanup procedures. Include this fact sheet in the CHP as a standard operating procedure (SOP).
- Review an EtBr Material Safety Data Sheet (MSDS) and this EH&S fact sheet before handling the material.
- Wear eye protection and ensure that there is unobstructed access to an eyewash/shower unit in the work area.

- As with any chemical, to avoid ingestion do not eat or drink where EtBr is handled, processed, or stored.
- Always wash hands thoroughly after handling EtBr, even if gloves are used.

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- Use 20–100 ng DNA per band for samples containing one unique band or up to 500 ng per lane for samples containing multiple bands.
- Prepare DNA samples in a total sample volume of 15 µL for E-Gel® 48 Gels in deionized water or loading buffer (we recommend using a final loading buffer concentration of 10 mM Tris-HCl; 1 mM EDTA, pH 7.5; 0.005% bromophenol blue; and 0.005% xylene cyanol FF).
- Dilute samples containing >50 mM NaCl, >100 mM KCl, >10 mM acetate ions, >10 mM EDTA (high salt samples) 2- to 20-fold in deionized water, TE, or loading buffer, in a final volume of 15 µL.

Preparing Loading Plate(s)

1 DNA Away and Ethanol the workstation.

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Thaw and spin down samples.

3 Add **13 µL** of molecular grade water to the first 48 wells of a 96-well skirted plate.

4 Set multichannel pipette to **35 µL** (more than 30uL in each PCR plate well).

4.1 Combine A, B, C into B and E, F, G into F.

> **Note:** Do not touch NTC lanes!

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Spin down PCR plate (combined) with a temporary seal.

6 Add **2 µL** from B, D, F, H (sample and NTC) to skirted plate with water (do this for each PCR sample or plate).

1. Combine B into A
1. Combine D into B
1. Combine F into C
1. Combine H into D

Making Ladder Dilution

7 **Make the ladder dilution:** 10uL water + 5uL 1Kb Plus DNA Ladder (green top)

For One Gel: (10uL x 4 wells) = **40 µL** water + (5uL x 4 wells) = **20 µL** ladder stock

> **Note:** Multiply each value above by the number of gels that are planning on running.

Loading E-Gel 48-Well 2% Agarose Gels

8 **Load the EGel:**

8.1 15 uL sample/water dilution mix to odd rows (top and bottom)

8.2 15 uL NTC/water dilution to even rows (top and bottom)

8.3 15 uL ladder dilution to each of the wells marked “M” in each of the 4 corners

Running E-Gel 48-Well 2% Agarose Gels 18m

18m

9 Run EGel in the E-Base Invitrogen for **00:18:00** .

Imaging E-Gel 48-Well 2% Agarose Gels 10m

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Take EGel to a Gel Imaging System: Gel Doc XR+ System

10.1 Turn on EpiWhite, load EGel into the bottom tray and close.

10.2 Open computer program and select File - Gel Doc XR.

10.3 Open top cabinet and line Gel up in the viewfinder.

10.4 Turn off EpiWhite. Click AutoFocus, and then turn on Trans UV.

10.5 Once the image is focused and bright, click freeze and turn Trans UV off.

10.6 Print the image at 110% and save the image both as the original file and as a JPEG.

10.7 Clean the stage EGel was resting on with Windex and throw Gel away in non-RCRA waste.