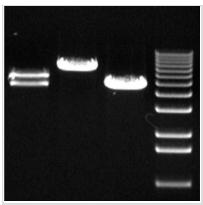

Jun 03, 2022

# 2% Agarose Gel

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protocol .

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Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA or proteins in a matrix of agarose, one of the two main components of agar. The proteins may be separated by charge and/or size (isoelectric focusing agarose electrophoresis is essentially size independent), and the DNA and RNA fragments by length. Biomolecules are separated by applying an electric field to move the charged molecules through an agarose matrix, and the biomolecules are separated by size in the agarose gel matrix.

An Agarose gel is easy to cast, has relatively fewer charged groups, and is particularly suitable for separating DNA of size range most often encountered in laboratories, which accounts for the popularity of its use. The separated DNA may be viewed with stain, most commonly under UV light, and the DNA fragments can be extracted from the gel with relative ease. Most agarose gels used are between 0.7–2% dissolved in a suitable electrophoresis buffer.

sybr_safe_dna_gel_stain_man.pdf

George Testo 2022. 2% Agarose Gel. **protocols.io** https://protocols.io/view/2-agarose-gel-cac7sazn

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**Reagents**

- 1 x DI Water
- 1 x bottle of TAE (or TBE)
- 1 x bottle of Agarose Powder
- 1 x bottle of SYBR Safe
- 1 x bottle of 6x Loading Dye

**Supplies**

- Green temporary seal(s)
- Foil seal(s)

**Equipment**

- 10uL pipette, tip box, & tips
- 20uL pipette, tip box, & tips
- 200uL pipette, tip box, & tips
- 1000uL pipette, tip box, & tips
- Serological pipette & 10mL tip

SYBR™ Safe DNA gel stain showed no or very low mutagenic activity when tested by an independent, licensed testing laboratory, and this stain is not classified as hazardous waste under U.S. Federal regulations. The safety testing included 3 well-established mammalian cell–based tests (Table 2, page 3), a battery of well-established Ames-test bacterial strains (Figure 2, below), and extensive testing for environmental safety (Tables 3 and 4, page 3). Use care when using this reagent and dispose of the stain in compliance with all pertaining local regulations.

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> TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

You may store the SYBR™ Safe DNA gel stain at any temperature between 2°C to 25°C. SYBR™ Safe in DMSO freezes at low temperatures; therefore, the product must be completely thawed and mixed before using. Repeated freeze-thawing has minimal impact on product performance.

## Pouring a Standard 2% Agarose Gel 21m

1 Measure 1g of Agarose.

2 Mix agarose powder with **49mL of DI water** and **1mL of 50x TAE** in a microwavable flask.

3 Microwave for **1-3 minutes** until the agarose is completely dissolved. 1m

**Note:** Do not overboil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel. Many people prefer to microwave in pulses, swirling the flask occasionally as the solution heats up.

**Caution:** HOT! Be careful stirring, eruptive boiling can occur.

4 Let agarose solution cool down to about **50°C** for about **00:05:00** . 5m

5 

**OPTIONAL:** Add **5uL of SYBR Safe** to the flask.

**Note:** EtBr binds to the DNA and allows for visualization under ultraviolet (UV) light.

**Caution:** EtBr is a known mutagen. Wear a lab coat, eye protection, and gloves.

6 Pour the agarose into a gel tray with the well comb in place.

7 Place newly poured gel at **4 °C** for **00:15:00** or let sit at room temperature for 30 minutes, until it has completely solidified. 15m

**Note:** Pour slowly to avoid bubbles which will disrupt the gel. Any bubbles can be pushed away from the well comb or the sides/edges of the gel with a pipette tip.

## Loading Samples and Running an Agarose Gel 21m

8 Add loading buffer to each of your ladders and DNA samples.

8.1 Make your Ladder Dilution:

Make your Ladder Dilution: **Molecular Grade Water + 10uL of 1kb Ladder**

8.2 On a small sheet of parafilm, pipette mix at least **10 µL of ladder or sample** with **2 µL of 6x Loading Dye** for a total volume of **12 µL** for gel loading. For lower concentrations of DNA, pipette mix at least **15 µL** of ladder or sample with **2.5 µL** of 6x Loading Dye.

9 Once solidified, place the agarose gel into the gel box (electrophoresis unit).

10 Fill gel box with 1x TAE (or TBE) until the gel is covered.

11 Carefully load a molecular weight ladder into the first lane of the gel.

12 Carefully load your samples into the additional wells of the gel.

Run the gel at 80-150V until the dye line is approximately 75-80% of the way down the gel. A

13 typical run time is about 1-1.5 hours, depending on the gel concentration and voltage.

**Note:** Black is negative, red is positive. The DNA is negatively charged and will run towards the positive electrode. ALWAYS RUN TO RED.

## Analyzing Your Gel 21m

14 Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.

**Note:** If you did not add EtBR to the gel and buffer, place the gel into a container filled with 100mL of TAE running buffer and 5uL of EtBr, place on a rocker for 20-30 minutes, replace EtBr solution with water and destain for 5 minutes.

15 Using any device that has UV light, visualize your DNA fragments. The fragments of DNA are usually referred to as “bands” due to their appearance on the gel.

**Note:** If you will be purifying the DNA for later use, use long-wavelength UV and expose for as little time as possible to minimize damage to the DNA.

**Caution:** When using UV light, protect your skin by wearing PPE.