HOW TO MAKE A CAROLINA BLU™ GEL (0.7% agarose for DNA)

Wear goggles and hot gloves when handling hot agarose*

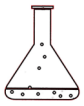
Materials:

- centigram balance
- weigh boats or paper
- bottle or flask, 3X volume of gel solution
- graduated cylinder for agarose solution
- hot gloves and goggles
- microwave oven or hot plate
- gel electrophoresis box & power supply
- 150 ml plastic beaker to hold buffer
- agarose powder
- 1X TAE buffer
- Carolina Blu #1 stain

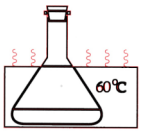
Consult chart on back for amounts of agarose and buffer to use and for useful hints

1. **Add:** ____ grams agarose to ___ milliliters buffer in large Erlenmeyer flask or bottle. (Lid **MUST** be loose before heating!)

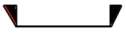
2. **Heat:** Until all particles are dissolved, ~30 sec to 1 min after solution boils. Mix by swirling flask or bottle several times during heating.



3. **To Cool or Store:** Keep flask in a 60°C water bath.

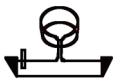


4. **Set up:** Place dams in gel box at each end of gel tray



5. **Add:** Carolina Blu #1: 2 drops to 50 ml agarose — then MIX.

6. **Pour:** 25 ml of agarose into the gel tray. Insert comb at negative (black) end for DNA.



7. **Cool:** Let gel harden 10 min.
   Add: 6 drops Carolina Blu #1 to 125 ml 1X TAE gel buffer.
   Pour: some buffer over top of gel.
   Remove comb & dams gently.

   If you choose to **store the gel before running:**
   Write your name on an acetate sheet and slide it under the gel.
   Store them in a Tupperware container or Ziploc bag with buffer covering all of the gels.
   Gels containing Carolina Blu should be used within 24 hours.

8. **Run:** Add the remaining buffer, load 15 µl samples, and run the gel at 100 volts.

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MAKING & STAINING GELS WITH CAROLINA BLU™

In DNA labs, 0.7% agarose gels are used. This low agarose % will allow the DNA to run faster, thus shortening the electrophoresis time to 30-45 min. Note that these gels will be more fragile than 1% agarose gels used in the Dye Lab.

MAKE THE AGAROSE SOLUTION

1. Wear goggles. Obtain a bottle with a **loosened cap** or an Erlenmeyer flask. The container's volume should be about 3X the volume of the solution to prevent boiling over.
2. Use Table 1 to calculate the amount of agarose and buffer you will need. Add the agarose powder to the buffer and mix.

   Note: Wear hot gloves. Agarose will boil over quite easily! ***Beware of steaming hot agarose.!***
3. To dissolve the agarose, heat the mixture to boiling in microwave or on a hot plate for ~30 seconds to 1 min after the mixture begins to boil. Swirl the bottle occasionally as it heats.
4. Swirl the bottle to see if agarose is dissolved. If any clear floating particles are visible, heat it for another 30 seconds and check again.
5. When the agarose is dissolved, add the appropriate amount of Carolina Blu #1 to the solution and swirl to mix. See Table 1. *Note: Carolina Blu must be added* ***after*** *the agarose has dissolved!*
6. Place the hot container in a 60°C water bath (Rival Hot Pot works well) or oven to hold the melted agarose at the right temperature for pouring gels throughout the day.

Table 1

No. gels @ 25 ml each	agarose	1X TAE	HEAT & DISSOLVE THEN ADD CAROLINA BLU #1	Carolina Blu #1
2	0.35 gm	50 ml		80 μl (2 drops)
4	0.7 gm	100 ml		160 μl (4 drops)
10	1.75 gm	250 ml		400 μl (10 drops)

POUR THE GEL

7. You can measure 25 ml or just fill the tray, with dams in, to the edge with agarose. You do **not** have to cool the agarose to pour the gel in the Horizon 58 gel boxes. They can take the heat. You do, however, need to pour the agarose *slowly* so that it does not leak under the dams.

Table 2

Amount of Carolina Blu #1 to add to 1X TAE buffer for running DNA gels

# of gel boxes	1X TAE Buffer volume	Carolina Blu #1 volume
1	125 ml	240 μl (6 drops)
8	1000 ml	1.9 ml (48 drops)

STAIN & DESTAIN THE GEL USING CAROLINA BLU #2 FINAL STAIN

8. **Stain:** Following the electrophoresis, place the gel into a staining tray. Cover the gel with Carolina Blu #2 and allow to sit for 15 minutes. Agitate gently if possible. Pour the stain into a container (it can be reused).
9. **Destain:** Cover the gel with distilled water. (Tap water contains chloride ions that may partially remove the stain from DNA bands.) Occasionally, gently agitate the gel. Change the water 3-4 times over the course of 30-40 minutes. The gel can be left in a little water to destain fully. If the gel destains too much, you can restain it. For best results, monitor the gel during destaining.
10. **Store:** Once destained, the gel can be covered in plastic wrap, placed in a storage bag, or left in the staining tray covered in plastic wrap and stored in the refrigerator. Stored in this manner, bands are visible for 6-8 weeks.

    Note: With these stains and gels, you need about 1.0 μg DNA per lane to see the bands