Formamide gel electrophoresis

Contributed by Jim Anderson

Formamide gel mix:

	100 mL mix	500 mL mix (enough for 5 gels)
dd H_2O	15 mL	75 mL
Formamide	32 mL	160 mL
Urea	3.6 g	168 g
10X TBE	10 mL	50 mL
AccuGel (19:1)	17.5 mL	87.5 mL
Total	**100 mL**	**500 mL**

Be sure to add formamide and stir in the fume hood. Add AccuGel with 25 mL pipettes dedicated to acrylamide use only. Store in the refrigerator.

Gel preparation:

1. Pour about 100 mL of Formamide gel mix into a 150 mL beaker with a small stir bar.
2. When apparatus is all assembled, add 1ml of 10% Ammonium Persulfate. (Make fresh if it is more than 2 weeks old). Add 40 µL TEMED. Stir gently for a few seconds to mix.
3. Draw the solution into the casting syringe. Plug it into the hole in the bottom of the apparatus. Tilt the apparatus slightly while pouring to prevent the formation of bubbles at the comb.
4. Allow the gel to set at least 45 minutes before using.

Preparation of the glass plate:

1. Wash plate thoroughly with Alconox and clean it with 95% Ethanol.
2. Prepare fresh binding solution by adding 5 uL Bind Silane and 5 uL glacial acetic acid to 1 mL of 95% ethanol.
3. Wipe the clean short glass plate using a Kimwipe tissue saturated with freshly prepared binding solution.
4. After 5 min. wipe the plate with 95% ethanol using gentle pressure (repeat this wash 5 times).
5. Change gloves.

Preparation of the chamber plate:

1. Wash thoroughly the chamber plate with Alconox and clean it with 95% ethanol.
2. Treat plate with a Kimwipe saturated with Rain-X.
3. After 5 min. buff the plate with a Kimwipe moisten with dd H_2O, then dry through with a fresh Kimwipe.

To run the gel:

1. Place the gel cast in the electrophoresis apparatus (Bio-Rad Sequencing Cell).
2. Pour 2L of 1X TBE buffer to the apparatus.
3. Pre run the gel for 15-30 min (50°C and 110 watts).
4. Clean the loading front of the gel with a syringe filled with 1X TBE.
5. Load the samples and run the gel for about 90 minutes.

Silver Staining Procedure:

Prepare the following solutions:

1. Fix/Stop solution (10% glacial acetic acid): Mix 1.8 L dd H_2O and 200 mL glacial acetic acid.
2. Developing solution: combine 2 L dd H_2O, 60 g Na2CO3 and chill to 4°C. Add 3 mL 37% Formaldehyde, and 400 µL Sodium Thiosulfate (10 mg/mL) after chilling just before use.
3. Staining solution: Mix 2 L dd H_2O, 2 g $AgNO_3$, and 3 mL of 37% Formaldehyde.

Procedure:

1. After electrophoresis, carefully separate the plates.
2. Place the gel plate in a plastic tray containing 2 L fix/stop solution and gently agitate for 20 minutes.
3. Decant the fix/stop solution for later use.
4. Rinse the gel with dd H_2O 3 times (2 minutes each) with gentle agitation.
5. Add staining solution and shake the gel gently for 30 minutes.
6. Rinse the gel BRIEFLY (about 10 seconds) with dd H_2O.
7. Add prechilled developer solution to the gel and shake until the bands become visible.
8. Dump the developer solution, add the fix/stop solution and gently shake the gel for 2 minutes. This will terminate the developing reaction.
9. Rinse the gel twice with dd H_2O (2 minutes each).
10. Dry the gel using air drying. Leave the gel overnight to dry.
11. Take a picture of the gel on a light box using Automatic Processor Compatible (APC) film (Promega Catalogue #Q4411).