

## Agarose Gels

### Procedure

Weigh out the required quantity of agarose; 1g per 100 ml = 1% gel

Place it into an Erlenmeyer flask.

Add the appropriate quantity of concentrated buffer stock solution.

Bring the volume up to the final volume.

Microwave on high just until you start to see the appearance of boiling. Stand there the entire time; i.e., don't walk away.

Remove the flask.

Carefully, swirl the agarose mixture. If it is superheated it will boil over when you swirl it and you will get scalded.

Return the flask the microwave.

Microwave it again until you see boiling.

Repeat the swirling.

Continue this cycle until there is no sign of any solid bits of agarose remaining.

Add Ethidium bromide to the liquid gel (1-5 $\mu$ l of a 10mg/ml stock solution per 100 ml of agarose gel).

Allow the agarose to cool to 50°C to 65°C. If you don't it will warp the gel boxes.

### Notes:

For most applications use a 1% agarose gel. For very low molecular weight DNA (<50 base pairs) use a 4% agarose gel, or even better, use an 8-10% acrylamide gel (see AcrylamideGels(DNA)). For very high MW DNA (>50 kb) use a 0.2 to 0.4% agarose gel. (0.2% will need to run in a cold room).

There are two main buffer systems for agarose gels. TAE and TBE. TBE has the advantage of having a very high buffering capacity whereas TAE does not. DNA fragments of 0.5 to 2.0 (or less) can be resolved quite well in TBE at 80 or so volts. Larger fragments are best resolved in TAE using a ~ 10 cm gel at very low volts (i.e., 30 V for ~18h). The general rule of thumb is to run the gel so that the final V-h = 500. For example 35V for 14h = 490 V-h works great to give beautiful Southern Blots. But when you're in a hurry and all you need to do is see a band use TBA and crank it up to 100 V for 2h. Just don't melt the gel because the higher the volts the greater the heat generated. Also realize because TAE does not have much buffering capacity that if there is no exchange between the anode and cathode buffers, frequently a very strong pH gradient will develop and your gel will melt down! A great advantage of TAE is that you can make a 50X stock solution that will last a long time. TAE is also the preferred buffer when you are isolating DNA fragments from gels to use in subsequent enzymatic reactions, ligations (i.e., cloning) labeling, etc.

## Buffers

### 50X TEA (TAE)

	1L
(1) Tris base	242g
(2) Na-Acetate	136g
(3) Na <sub>2</sub> EDTA	18.6g
(4) H <sub>2</sub> O	
(5) Adjust pH to	8.2 with glacial acetic acid
(6) Bring final volume to	1L
(7) Filter (optional).	
(8) Store at RT.	

### 10X TBE

	1L
(1) Tris base	108g
(2) Boric Acid	55g
(3) EDTA	7.45g
(4) H <sub>2</sub> O to	1L

(no need to pH)

- (5) Filter (optional).  
(6) Store at RT.

I generally filter my TAE and TBE through a 0.45µm Millipore membrane. This is optional for most applications but recommended for more sensitive applications; i.e., sequencing gels and some protein gel uses.

### Ethidium Bromide

is a mutagen and must be handled with care. To make a 10mg/ml stock weigh 1.0g and add it to 100ml dH<sub>2</sub>O add a stir bar and let it dissolve several hours to overnight. Store in a brown bottle.

### Gel loading buffer dye (good for just about all applications)

	10ml
Glycerol	7.5ml
Bromophenol Blue	~2mg
Xylene Cyanol	~2mg
1M Tris	100µL
0.5M EDTA	20µL
10% SDS	200µL
H <sub>2</sub> O	2.2ml

The general rule of thumb is to use 1µL of gel loading buffer dye per 5µL of sample.