

3:1 Agarose Gel Preparation Protocol

1. To make gels with agarose concentration less than 2%:

(1) Use a flask that is 2 to 4 times the volume of the solution being prepared.

(2) Add the correct amount of dry agarose to a measured quantity of electrophoresis buffer.

(3) If use a boiling water bath :

- To melt the agarose, simply by heating the slurry in a boiling water bath, bring the solution to a boil and allow it to boil for 5-10 minutes stirring continuously, until the agarose dissolves completely.

If use a microwave oven :

- To melt the agarose in solutions of less than 2%, heat the slurry in microwave oven on high power setting until it starts to boil.
- Allow the solution to boil for 1 min or until the solution is clear and all particles are dissolved.
- Remove the flask from the microwave oven, and gently swirl to mix the agarose solution.

Use caution when handling as solution may be extremely heated.

(4) Cool the solution to approx. 60°C before pouring.

2. To make gels with agarose concentration greater than 2%:

(1) Use a flask that is 2 to 4 times the volume of the solution being prepared.

(2) Add the correct amount of dry agarose to a measured quantity of electrophoresis buffer.

(3) Heat the slurry in a microwave oven on a medium power setting until it starts to boil.

(4) Remove the flask from the oven and gently swirl to resuspend the gel particles.

(5) Reheat the solution on a medium power setting until it starts to boil again.

(6) Afterwards, remove the flask from the microwave and gently swirl.

If the agarose did not completely dissolve, reheat the solution again.

(7) Cool to approx. 70°C before pouring.

Separation of DNA in agarose

Size range (bp)	500 – 1,000	100 – 500	10 – 100
Agarose in gel (%) 1 × TAE Buffer	3.0	4.0	6.0
Agarose in gel (%) 1 × TBE Buffer	2.0	3.0	5.0