

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