AGAROSE GEL ELECTROPHORESIS

For separating DNA fragments between 600 bp and 5 kb we use 1% agarose gels. Higher resolution in the 300 bp - 1 kb range can be achieved using 1.5% agarose. We have different grades of agarose for different applications. For analytical work use BioRad LE or BioRad high strength analytical grade. For recovering DNA from agarose using Geneclean use GTG agarose. SeaKem GTG is used for DNA fragments larger than 300 bp. For smaller fragments use Nusieve GTG and electro-elute or just melt it and use it melted. Nusieve GTG is a low melting point agarose which once melted will stay liquid at 37C. Nusieve is very expensive so use it only for applications where other agaroses will not work. Similarly, GTG agarose is 2-3 times more costly than analytical agarose. Use agarose for preparative work of fragments >300 base pairs. For fragments <300 base pairs use the method described on the next page.

To pour a gel add the required amount of agarose to 1 X TAE. For 100 mls of 1% agarose, add 1 gram to 100 mls of 1 X TAE. Melt the agarose by boiling in the microwave. You have to stand and watch it. For analytical agarose bring to a boil a couple of times, swirl in between and that should do. GTG agarose is harder to melt. After it is completely melted allow it to cool until it is warm but not hot to the touch (÷50C). After you have run your gel, cut out the piece you have used and place it inot ~100 mls of TAE buffer containing ethidium bromide.

Ethidium bromide is stored at 4C in solution at 5 mg/ml. It is a mutagen in vitro and therefore probably also in vivo. Therefore, handle ethidium bromide solutions with caution. Wear gloves at all times. To calculate the correct volume to add, divide your total buffer volume in mls by 20. The result is the number of ul of 5 mg/ml stock to add. For our example, 100 mls ö 20 = 5 .. add 5 ul. Add this same amount to any solution used for staining DNA.

For our gel boxes use the following amounts of agarose:

- Tyler large box 250 mls
- Fisher small box 40 mls
- BRL 90 lane large box ÷250 mLs small box ÷100 mls

TAE is: Tris Acetate EDTA

- 1 X .04 M Tris Ac
- .002 M EDTA
- 50 X 1 M Tris Ac for 1 litre 50 X
- 0.1 M EDTA 242 g Tris base pH 8.5
- 57.1 ml glacial acetic acid
- 37.2 g EDTA