

## A procedure for the formation of agar salt bridges.

The purpose of an agar salt bridge is to provide an electrical connection to the bath solution while minimizing the transfer of ions or solute from the electrical environment.

Use the following procedure to create salt bridges. This procedure involves:

- 1) formation of bridges
- 2) preparing the agar
- 3) loading the bridges with agar
- 4) storing the bridges for later use

### Formation of bridges

Bridge structures can be formed from any tubular material which is both chemically and electrically inert. Many researchers use either polyethylene or glass tubing. Due to its structural rigidity we recommend glass capillary tubing with an inside diameter of 0.86 mm. Use of larger diameter tubing can increase the transfer of material across the bridge while smaller diameter tubing can increase the series resistance of the conducting pathway. The proper choice of tube diameter will depend on your application and electronics. We suggest that you start with our recommendation and make changes as necessary.

The goal is to create a U-shaped tube which will bridge the barrier from the electrode well to the preparation bath. While the geometry you will create is dependent on your chamber, a reasonably close fit will minimize movement of the bridge and will prevent the salt bridge from interfering with your optics, electrodes, perfusion system, etc.

Capillary tubing can be easily bent into the desired shape by heating it in the flame of a Bunsen burner. However, take care to not overheat the tubing as the glass will completely melt and occlude the conducting pathway. A sufficient number of capillaries should be prepared in advance such that you will have between 20 and 50 working bridges at the end of this procedure. The ends of the U-shaped capillary can be trimmed to any desired length with a wire cutter. Use caution to not generate long cracks in the capillary while cutting.

### Preparing agar

An agar solution is formed by heating a mixture of 2-5% agar in 1M KCl (w/v). A final volume of 3 ml is sufficient to fill 50 bridges. This mixture should be covered and gently heated, with stirring, on a hotplate until the agar is completely dissolved (solution becomes clear) and bubbles are just beginning to form.

### Loading bridges

Once the agar has been properly prepared the bridges should be immediately filled. This can be easily achieved by dipping one end of the U-shaped tube into the hot agar mixture. Capillary action will draw the agar into the tube, filling it completely. Monitor this process to prevent the formation of bubbles within the capillary. Alternatively, bridges can be dunked whole into the hot agar to fill them. However, the probability of forming a bubble within the tubing is greatly increased since agar will simultaneously fill the tubes from both ends.

Agar filled tubes should be immediately placed in fresh 1M KCl to prevent shrinkage of the agar during cooling.

## **Storage**

When cool, remove any excess agar from the outside of the bridges and examine for bubbles. Usable bridges should be placed in fresh KCl solution for long-term storage. Bridges may be covered and safely stored in the refrigerator for up to 90 days.

Salt bridges can be discarded after each use or reused several times.