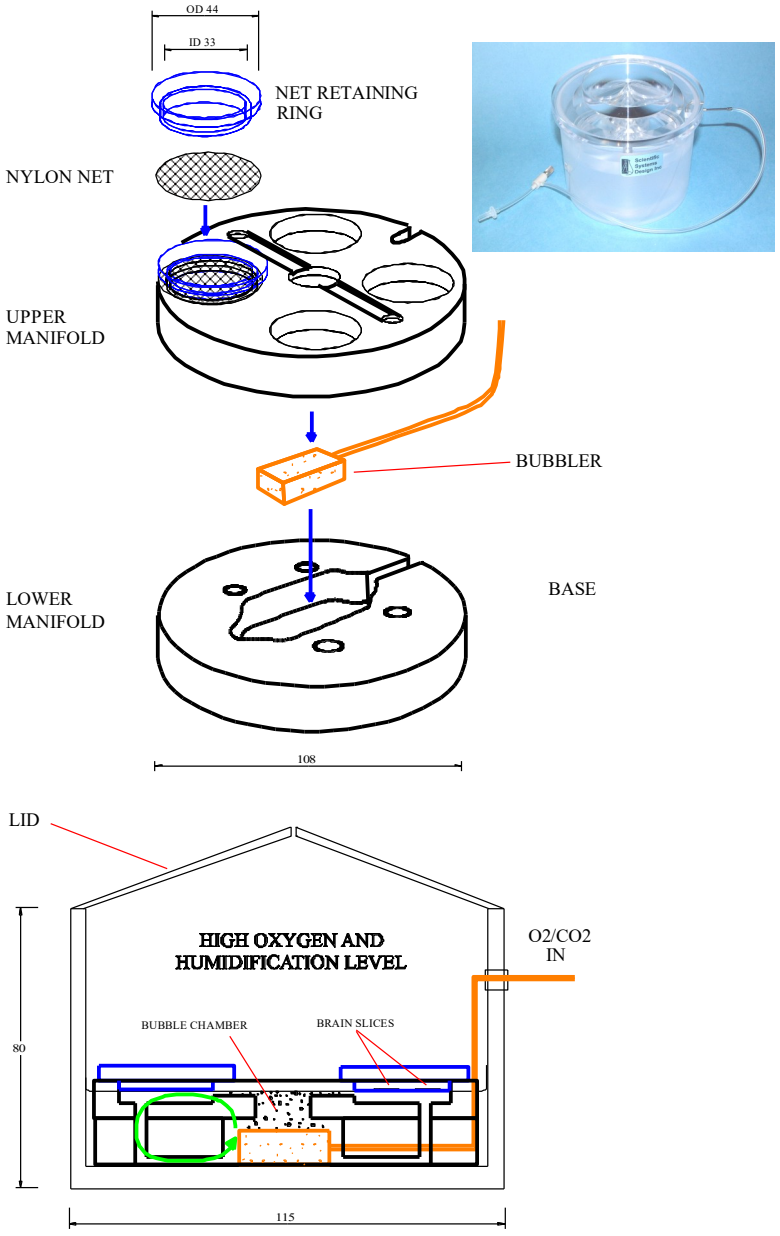


Schematic arrangement of BSK5-4 and location in purpose designed trough with conical profiled lid. Profile shows two of the four rings in cross section. Oxygen bubbles rise in the center carrying aCSF up and across beneath the surface of the slices, solution is re-directed towards the bubbler to create continuous circulation (green arrow). Approximate dimensions are in mm.

Scientific Systems Design Inc

1220 Steeles Ave East
Unit G6 1006
Milton
Ontario L9T 6R1
CANADA

Phone:1 905 608 9307
ssd@scisys.info
www.scisys.info

Innovative Engineering for Science

BRAIN SLICE KEEPER

BSK5-4

Interface or Submerged Mode

BRAIN SLICE KEEPER

CAUTION !

YOUR BRAIN SLICE KEEPER IS A PRECISION ENGINEERED TOOL FOR SCIENTIFIC RESEARCH. PLEASE TAKE A FEW MINUTES TO FAMILIARISE YOURSELF WITH THE KEEPER AND READ THROUGH THIS SHORT MANUAL BEFORE ATTEMPTING TO USE IT. DO NOT USE ALCOHOL OR SIMILAR SOLVENTS IN ANY CONCENTRATION ON ANY PART OF THE KEEPER SINCE AS WITH MOST ACRYLICS, IT MAY FRAGMENT OR DEVELOP HAIR-LINE CRACKS.

OPERATION

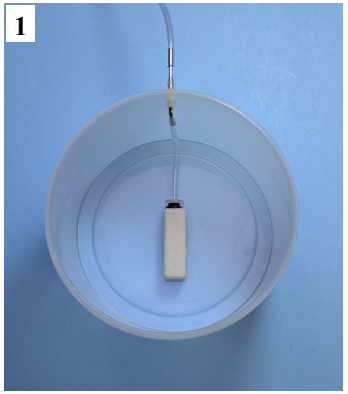
The BSK5-4 Brain Slice Keeper has been designed to pre-incubate a large number of brain slices prior to transfer into recording chambers. Based on the BSK4, it consists of four closely fitting acrylic rings, installed into four holes on an upper manifold. The close fitting rings allow a removable sheet of nylon netting to be wedged and held in place. In this slice keeper, the slices can optionally be maintained either at an interface between the incubation medium and a humidified, high oxygen atmosphere within the holding vessel or fully submerged. Furthermore the slices can be maintained on a flat porous membrane (eg *Millicell-cm, 0.4um Cat.# PICMORG50 from Millipore) placed on the nylon netting in order to maintain the underside of slices perfectly flat for subsequent experiments utilizing microscope techniques.

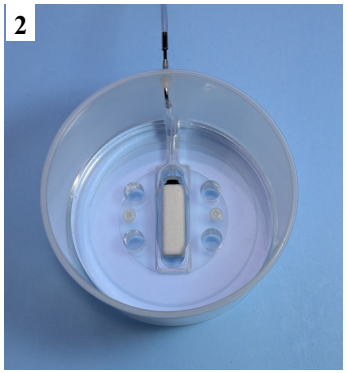
FEATURES

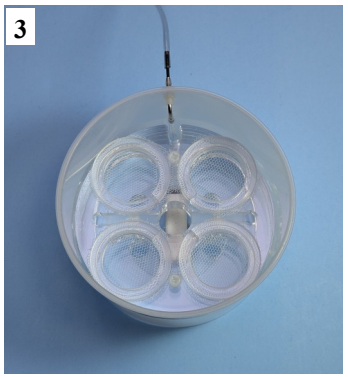
* *Slices maintained for many hours either in interface or submerged mode with incubating media*
* *Four separate rings allow separation of different types of preparations*
* *Slices supported on a quick change nylon net with optional porous membrane*
* *Modular design simple to set up and maintain*

In operation, the separate, lower manifold is first placed into the bottom of the vessel and the bubbler placed centrally in the purpose designed opening such that it rests firmly on the base of the vessel centre. Next, the manifold carrying four rings is located on top of the lower manifold, aligning the slot for the incoming tubing for the bubbler. For use in interface mode, the holding vessel is partially immersed with aCSF by filling to the level of the netting. The purpose-designed holding vessel is supplied with a 95% O2, 5% CO2 gas mixture via the bubbler. The bubbles rising from the base manifold saturate the aCSF and provides constant circulation of medium to the slices from the underside. Typically slices are placed on lens tissue which rests on the nylon nets or optionally on porous membrane discs attached to the netting. Bubbles rising in the central area are thereby kept from being trapped under the slices, ensuring a continuous circulation of medium. Slices remain viable for many hours in these conditions. In submerged mode, the aCSF is filled to at least 2mm above the top of the four 'C' rings'. The BSK5 together with the holding vessel can be easily placed into a water bath for regulating the incubating temperature as desired. A lid with a conical profile ensures that condensation drops do not fall directly on top of the slices and maintains high oxygen convcentration in interface mode. The typical fluid volume with BSK5-4 is around 110ml in interface mode and 200ml in submerged mdoe.

*Micro-90™ is a Trademark of International Products Corporation USA







1) Position bubbler in center of the vessel. 2) Place lower manifold over the top of the bubbler. Check bubbler is completely flat on the base with lower manifold. 3) Place upper manifold containing the four slice keeper rings on top of the lower manifold, aligning the groove for the vertical gas inlet tube. Fill with aCSF (approx. 110ml for interface mode) so that the bottom of the netting is at the same level as the aCSF level. Use the conical profiled lid to maintain high humidity and oxygen concentration . For submerged mode fill 2mm above the four 'C' rings (approx. 200ml)

TEMPERATURE CONTROL

Temperature can be maintained by submerging the trough into a water bath set to the desired temperature. The heated water bath fill level should be about 15mm below the fill level of the BSK5 to prevent floating and instability. Bubbling within the BSK5 ensures circulation and uniform temperature of the incubating media around the slices.

MAINTENANCE

Alcohols should never be used on the acrylic manifold and rings of the slice keeper for cleaning purposes even at low concentrations because it dehydrates and produces hair-line cracks in acrylic plastics. Only the plastic trough can be cleaned with alcohols. The acrylic component can be cleaned with special laboratory detergents such as *Micro-90™ which completely rinses out. The bubblers must be removed when using such detergents. Heavy deposits of salts should be washed out with distilled water overnight and carbonate salts treated with mild acids such as citric acid. The most common contaminant is fungal growth, this can be avoided by washing out with distilled water and drying out completely at the end of each day. Hydrogen peroxide solution 30 Vols, 1/10 dilution is also an effective cleaning agent followed by overnight soaking in distilled water. If necessary the two nylon screws on each of the two components can be loosened a few turns to allow cleaning between the surfaces or disassembled completely if there is excessive ingress of growths.