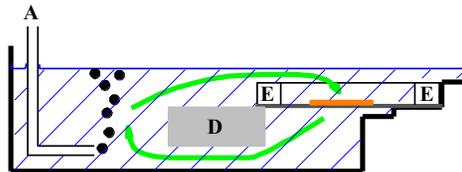
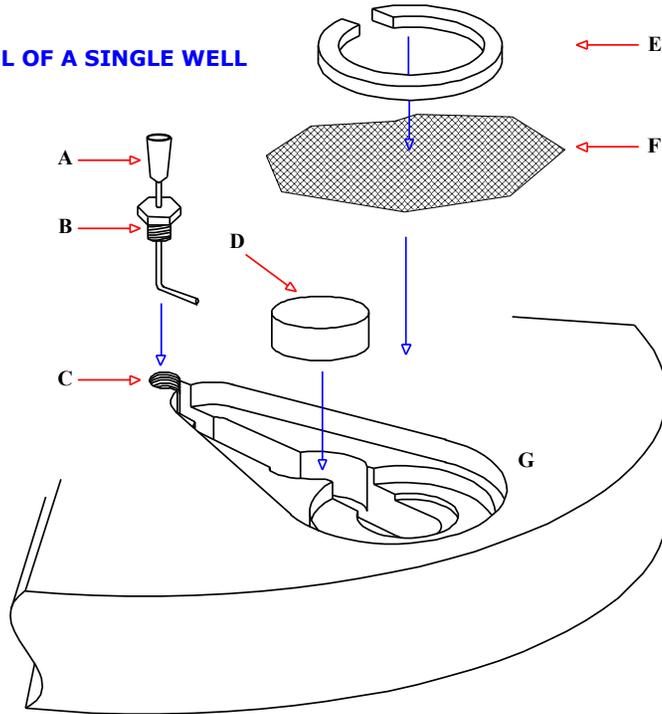


DETAIL OF A SINGLE WELL



Schematic arrangement of BSK6-6 with detail of a single well including a cross-section view.

Construction: A blunted 18G hypodermic needle [A] is inserted into a plastic fitting [B] and screwed into the chamber at [C]. The blunted tip is bent at 90° and used to bubble the gas mixture which creates a flow of solution towards the slice located on the nylon netting, which is in turn held by a tightly fitting 'C' ring [E]. The flow path continues under an acrylic plug [D] returning back to the bubbling region to complete the cycle (green arrows in lower drawing) The acrylic plug is normally held in place by the 'C' ring but can be lifted out when cleaning the slice keeper. The nylon netting [F] is placed over the well [G] and the 'C' ring [E] pushed down over it and the excess is removed by cutting with a new scalpel blade or small scissors.



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Innovative Engineering for Science

BRAIN SLICE KEEPER

BSK6

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. DO NOT AUTOCLAVE AS HEAT CYCLES MAY INTRODUCE STRESS CRACKS IN ACRYLIC.

The BSK6-6 Brain Slice Keeper has been designed to pre-incubate six separate sets of brain slices in solution volumes of 2ml (BSK6-6-2) or 4ml (BSK6-6-4) prior to transfer into recording chambers. This enables slices to be pre-loaded with experimental test solutions that are too expensive for large volume dilutions. It consists of a large acrylic disc into which is machined six completely independent wells. Each well has a closely fitting acrylic ring between which is wedged a removable sheet of nylon netting. The design of each well is such that when bubbled by an attached needle, a stream of oxygen bubbles introduced from the side are made to circulate the solution from the top downwards on to the net whilst also saturating the solution with oxygen. A channel below the net re-circulates the solution and exerts a downward force on the slices holding them down on the net.

FEATURES

- * Minimum 2ml or 4ml per incubating well for expensive test solutions
- * Modular design allows efficient cleaning between experiments
- * Slices supported on a quick-change nylon net
- * Simple to set up and maintain

OPERATION

In operation, each of the BSK6-6 wells is filled with ACSF until the 'C' ring insert is totally immersed at least 1mm above the rim. The fill volume will be approx 2ml or 4ml depending on the model. Each well is supplied with a separate feed of 95% O₂, 5% CO₂ mixture via a needle submerged at one end of the well. The feed of the gas mixture to each well is from six fine flow gas regulators, supplied with the slice keeper. These in turn are supplied from a 1-into-6 gas input manifold also supplied with the chamber. Before introducing slices the wells are over-supplied with oxygen to saturate the incubating media. After a few minutes the oxygen flow rate is reduced and the BSK6-6 is ready to accept one or two slices per well which are placed on the net. The optimal gas flow rate to each needle is approx 120 bubbles in 30 seconds which can be determined by observing through a dissecting microscope and adjusting each needle valve. Slower gas flow rates may be insufficient for achieving circulation and oxygenation whilst faster rates may cause too much turbulence and result in slices moving. Nylon netting supplied with the slice keeper is inserted in each well by pressing the 'C' ring over a large sheet until a section is wedged



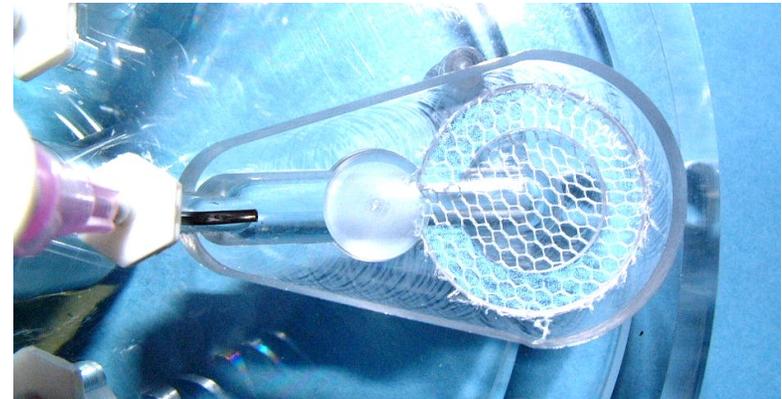
BSK6-6 Brain slice keeper with rings and net in place and with gas supply needles connected to fine gas flow valves supplied from 1-into-6 manifold (inset).

oxygenation whilst faster rates may cause too much turbulence and result in slices moving. Nylon netting supplied with the slice keeper is inserted in each well by pressing the 'C' ring over a large sheet until a section is wedged

tightly. The excess is then cut with a new scalpel blade or small scissors. After several uses the nylon net can be replaced by pulling the acrylic 'C' ring with the included hooked tool. When a new slice keeper is utilised, the behaviour of fluids on the acrylic surfaces will be hydrophobic and flow may not be smooth. By bathing each well in aCSF (minus glucose) for two days the acrylic surfaces will bond with salts (as acrylic contains a very small amount of water molecules) and the surface will become more hydrophilic aiding smoother flow at the edges. This operation may be needed each time the chamber is cleaned with *Micro-90™ (see below).

MAINTENANCE

Alcohol should never be used on the acrylic manifold and rings of the slice keeper for cleaning purposes even at low concentrations because it de-hydrates and produces hair-line cracks in acrylic plastics. Acrylic components can be cleaned with special laboratory detergents such as *Micro-90™ which completely rinses out. 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, in 1/10 dilution is also an effective cleaning agent followed by overnight immersion in distilled water.



View of one well with acrylic 'C' ring wedging removable nylon net. Gas bubbler needle is on the left side.

Specifications:

Typical volume of solution inside chamber: 2ml (BSK6-6-2) or 4mls (BSK6-6-4)
Overall Keeper diameter: 130 mm
Chamber height: 65 mm
Usable net diameter in each well: 12.5 mm
Supplied with six fine flow gas regulators and an extraction tool for 'C' rings to replace nylon netting

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