



Technical Note:

Glass Cloning Cylinders. Recommendations for Use.

Introduction

There are several approaches available to allow the isolation and development of a population of cells derived from the descendants of a single cell. The selection of the appropriate method is dependent on the type of cell to be isolated, and the techniques used to grow the cells.

One of the simplest methods of isolating and development of a population of cells is by the use of a cloning cylinder. Cloning cylinders are designed to encircle the cells of interest and allow them to be harvested for further culture work, without negatively impacting the growth of other cell populations on the same culture plate.

The sterile glass cylinders are sealed to the petri dish with sterile high vacuum silicone grease to prevent leakage.

The main advantage of this method is the chosen cells have already demonstrated viability and have grown successfully.

The main disadvantage is that there is no guarantee that the isolated colony originated from a single cell.

Recommended Procedure for Use

- Sterilize the cloning cylinders by autoclaving at 121°C for between 20 and 30 minutes.
- Allow to cool completely before use.
- Select the colonies to be isolated using either a dissection or a phase contrast microscope.
- Remove and discard the growth media and rinse the plate twice with PBS to remove any floating cells
- Using sterile curved forceps, pick up a sterile glass cloning cylinder. Gently press the flat bottom of the glass cylinder onto smooth sterile high vacuum silicone grease and remove quickly with sudden upward motion.
 - When done properly this provides an even distribution of grease on the bottom of the glass cylinder.
- Set the glass cylinder over the selected colony inside the petri dish.
 - Press the cylinder down evenly with the forceps.
 - Make sure to press downward evenly to avoid gaps between the glass cylinder and the petri dish, which can cause the grease seal to leak.
 - Be very careful not to slide the cylinder across the colony to avoid smearing the silicone grease over the cells as this will prevent trypsin from contacting the cells.
- Add approx. 0.2mL of 0.25% trypsin or dilute EDTA to the cloning cylinder, but do not overflow the cloning cylinder.

- Incubate the culture dish at 37°C for between 2 and 5 minutes until the cells have begun to detach from the dish bottom.
- Aspirate the trypsin from the cloning cylinder and add growth medium to the cylinder.
- Gently remove the cells with a Pasteur pipette or micropipette from the cylinder and transfer to a suitable culture vessel and add appropriate growth media.
- **NOTE:** Diluting the cells too much at this stage may result in slow growth or no growth at all.
- Incubate the cells as per established conditions..

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